# Chemical Information Review Document

# for

# Deoxynivalenol
# [CAS No. 51481-10-8]

**Supporting Nomination for Toxicological Evaluation by the National Toxicology Program**

**March 2009**


*Prepared for:*
National Toxicology Program
National Institute of Environmental Health Sciences
National Institutes of Health
U.S Department of Health and Human Services
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## Abstract

Deoxynivalenol (DON) is one of several mycotoxins produced by certain *Fusarium* species that frequently infect corn, wheat, oats, barley, rice, and other grains in the field or during storage. It is a toxic byproduct of *Fusarium* head blight in grains and feeds. Potential human exposure to DON may occur from ingestion of products made from contaminated grains. It has been detected in buckwheat, popcorn, sorgum, triticale, and other food products including flour, bread, breakfast cereals, noodles, infant foods, pancakes, malt, and beer. There also is risk of occupational exposure by inhalation of pathogenic species of filamentous fungi and mycotoxins among farmers engaged in grain threshing. Human exposures to DON-contaminated grains have been reported to cause acute temporary nausea, vomiting, diarrhea, abdominal pain, headache, dizziness, and fever. In general, acute exposure of animals to DON resulted in decreased feed consumption (anorexia) and vomiting (emesis) while longer exposure caused reduced growth, and adverse effects to the thymus, spleen, heart, and liver. Reproductive and developmental studies in mice reported that DON caused axial skeleton abnormalities, exencephaly, and neural arch defects. *In vivo* studies also reported that DON induced mitogen-activated protein kinase phosphorylation and cytokine mRNA expression, as well as serum immunoglobulin (Ig) A and IgE levels. DON given on gestation days 6-19 decreased the mean gravid uterine weight in Sprague-Dawley rats as well as the average fetal body weight, crown-rump length, and ossification of fetal vertebrae. In male Sprague-Dawley rats, DON decreased seminal vesicle, epididymal, and prostate weights, as well as numbers of spermatid and cauda epididymal sperm. Serum concentrations of testosterone and follicle-stimulating and luteinizing hormones were modulated in a dose-dependent manner. DON also superinduced pro-inflammatory cytokine gene expression *in vivo* and *in vitro*. Gastric intubation of NIH mice with DON three times a week for 24 weeks induced lung adenocarcinoma and dysplasia of glandular stomach. DON induced DNA damage and chromosomal aberrations *in vitro* and *in vivo* but failed to produce gene mutations in Chinese hamster lung cells *in vitro* or in *Salmonella typhimurium*. Although mycotoxins are likely to occur simultaneously when found in grain and feed, synergism and antagonism have not been thoroughly studied. DON and nivalenol (NIV) are type B trichothecenes that often occur together, however, type A trichothecenes, such as T-2 toxin and diacetoxyscirpenol, or other *Fusarium*-produced mycotoxins (e.g., zearalenone, fumonisin B1, or fusarin C) also may occur in combination with DON and/or NIV. In general, type A trichothecenes tend to be produced in lower quantities than type B but are more toxic. DON has been reported to be the least toxic type B and T-2 toxin the most toxic type A trichothecene of those commonly detected. Evidence suggests that mycotoxins may also have synergistic effects with other fungal metabolites, metabolites originating from host plants, or compounds added to grain or feed sources. Studies with pure zearalenone have shown that it is less toxic than cereals naturally contaminated with zearalenone, indicating the presence of additional toxic substances in the matrix. In the *Kluyveromyces marxianus* yeast bioassay, DON plus NIV produced a synergistic toxic response, while DON plus T-2 toxin had an antagonistic effect. In mouse fibroblast L929 cells, a mixture containing DON, NIV, T-2 toxin, zearalenone, and fumonisin B1 produced greater inhibition of DNA synthesis than with treatment of each mycotoxin alone.

## Executive Summary

### Basis for Nomination

Deoxynivalenol (DON), a trichothecene mycotoxin produced by certain *Fusarium* species that frequently occur in corn, wheat, barley, rice, and other grains in the field, was nominated by the National Institute of Environmental Health Sciences for chronic toxicity and carcinogenicity studies and reproductive toxicity studies. The toxicological profile of DON is well described yet definitive long-term studies are generally lacking. In a previous two-year carcinogenicity study in mice, dietary administration of DON did not result in an increased incidence of neoplasms in males or females; in males, there was a decreased incidence of liver neoplasms, probably a result of lower body weights (Iverson et al., 1995 [PMID:8732880]). DON is genotoxic, immunosuppressive, teratogenic, and affects multiple reproductive endpoints. The widespread contamination of human foods and demonstrated toxicological activity of DON indicate the need for further studies to evaluate potential carcinogenicity and reproductive effects. Such definitive studies will also serve as index studies necessary for developing toxic equivalency factors (TEFs) for other tricothecene mycotoxins. Similar conclusions regarding the need for DON toxicological data have previously been articulated by several groups, including the Joint FAO/WHO Expert Committee on Food Additives and the European Commission Scientific Committee on Food (http://europa.eu.int/comm/food/fs/sc/scf/out44_en.pdf), and in a workshop organised by the ILSI Europe Natural Toxin Task Force (Larsen et al., 2004 [PMID:15342076]).

### Nontoxicological Data

General Information

DON is a toxic byproduct of *Fusarium* head blight and a contaminant of oats, corn, wheat, barley, rice, and other grains in the field. The general methods for the quantitative determination of DON are thin-layer chromatography, liquid chromatography, and enzyme-linked immunosorbent assay. The simultaneous determination of several trichothecenes can be done using gas chromatography with mass spectrometry, electron capture detection, or flame ionization detection, or by high-performance liquid chromatography with atmospheric pressure chemical ionization mass spectrometry. DON is considered a marker for other toxins in grains and feeds. In 1993, the Food and Drug (FDA) established advisory guidelines for DON in food and feed of: 1 ppm for finished wheat products for human consumption; 10 ppm in grain and its byproducts for ruminating beef cattle, cattle in feedlots older than four months, and poultry (not to exceed 50% of diet); and 5 ppm in grain and its byproducts for swine (not to exceed 20% of diet) and for all other animals (not to exceed 40% of diet). No standard, however, has been established for raw grain intended for milling and use in human food products.

Environmental Occurrence and Persistence

The occurrence of DON in the environment is mainly linked with *F. graminearum* and *F. culmorum*. The former species grows optimally at 25 ºC while the latter at 21 ºC. Mean DON concentrations in food commodities in several countries (e.g., Brazil, Canada, Germany, Sweden, the United Kingdom, and the United States) and reported in the literature were: 4-760 µg/kg for oats, 4-9000 µg/kg for barley, 1-5700 µg/kg for wheat, 13-240 µg/kg for rye, 3-3700 µg/kg for maize, and 6-5100 µg/kg for rice.

Human Exposure

Potential human exposure to DON may occur from its frequent contamination of oats, corn, wheat, barley, rice, and other grains in the field used in consumer food products. It has been detected in buckwheat, popcorn, sorghum, triticale, flour, bread, breakfast cereals, noodles, infant foods, pancakes, and malt and beer. Urinary excretion of DON has been correlated to cereal intake. A provisional maximum tolerable daily intake (PMTDI) of 1 µg/kg body weight (bw) was established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). In the Netherlands, 80% of one-year-old children exceeded the PMTDI, and 20% of them had twice the intake. Porridges were a significant source of exposure for the children.

There is a considerable occupational risk among farmers engaged in grain threshing due to inhalation of pathogenic species of filamentous fungi and mycotoxins. High concentrations of fungi were noted in both grain and grain-dust samples during the threshing of cereals by a combine harvester. DON concentrations ranged from 0.015-0.068 μg/g [0.051-0.23 μmol/kg] in wheat grain samples.

**Toxicological Data**

In 2001, the JECFA published a monograph summarizing the safety data of certain mycotoxins, including DON, found in food. In general, acute exposure to DON resulted in decreased feed consumption (anorexia) and vomiting (emesis). Short- or long-term exposure caused reduced growth, with higher doses affecting the thymus, spleen, heart, and liver. DON was metabolized by de-epoxidation and glucuronidation to less toxic metabolites. In studies of reproductive and developmental effects in mice, rats, rabbits, hens, and pigs, DON significantly decreased caudal epididymal weights in $B6C3F_1$ and IL-6KO mice. Additionally, it induced chromosomal aberrations *in vitro* and *in vivo* but failed to produce gene mutations *in vitro* in several assays (e.g., the Ames test and in Chinese hamster lung cells). This report makes no attempt to duplicate the data found in the review, but instead referrs the reader to the JECFA monograph at http://www.inchem.org/documents/jecfa/jecmono/v47je01.htm. Supplemental information for studies in laboratory animals (e.g., rats and mice) is presented in the following sections. None were found regarding acute or chronic exposure or initiation/promotion.

Human Studies

DON causes acute temporary nausea, vomiting, diarrhea, abdominal pain, headache, dizziness, and fever. Several epidemiological studies, mostly in Asia, have been conducted; the acute effects were attributed to consumption of *Fusarium*-contaminated grains and the presence of DON at 3-93 mg/kg [0.01-0.31 mmol/kg]. In 15 urine samples collected from female inhabitants of Linxian County, China, a high risk exposure region for DON and for esophageal cancer, and Gejiu, a low risk region, DON was detected in all samples; mean levels were 37 and 12 ng/mL [0.12 and 0.040 μM], respectively. In one-year-old Dutch children exposed to DON levels above the PMTDI, reductions in body weight and relative liver weight were estimated at 2.2 and 2.7% (confidence interval: 0.2-25%), respectively.

Chemical Disposition, Metabolism, and Toxicokinetics

In male $B6C3F_1$ mice orally given DON (25 mg/kg [84 μmol/kg] bw), DON was rapidly distributed to the following organs within 30 minutes after exposure (ng/g): kidney (5680) > heart (4530) > plasma (4430) > liver (3900) > thymus (3640) > spleen (2990) > brain (763). The concentrations were significantly higher in all the organs up to eight hours compared to control animals. Clearance followed two-compartment kinetics ($t(1/2)\alpha$ = 20.4 minutes, $t(1/2)\beta$ = 11.8 hours).

In male Sprague-Dawley rats administered a single dose of [$^{14}$C]DON (5 mg/kg [0.02 mmol/kg] bw) via gavage, the highest levels of radiolabeled DON (~291 ng/mL [0.982 μM]; 0.37% of dose) were detected in the plasma at eight hours. Of the administered dose, 37% was excreted in urine at 72 hours, and DON-glucuronide was the major urinary metabolite.

Acute Toxicity

One study in five-week-old BALB/c female mice orally gavaged with DON (10 mg/kg [34 μmol/kg] bw) or 30 μL saline (controls) followed by intranasal instillation of reovirus indicated that DON compromised resistance to respiratory viral infection. Ten days after instillation of reovirus expression of the reovirus L2 gene was 10-fold higher in lungs of DON-treated mice compared to saline controls. Interferon (IFN)-α, IFN-αβ receptor, and IFN-γ receptor gene expression was suppressed and induction of double-stranded RNA activated protein kinase R and oligoadenylate synthase 2 (type 1 INF-dependent antiviral genes) was inhibited. Bronchopneumonia caused by reovirus infection was clearly exacerbated by DON

and elevations in protein, monocyte chemoattractant protein-1, TNF-α, and inflammatory cells in the bronchioalveolar lavage fluids were markedly enhanced by the presence of DON.

In male rats, intraperitoneal (i.p.) injection of DON (5, 10, and 25 mg/kg [0.02, 0.04, and 0.84 mmol/kg]) affected platelets and white and red blood cells. It also induced necrosis of hepatocytes, lymphocytes, and pancreatic aciner cells assessed 3 and 24 hours post-injection.

Short-Term and Subchronic Toxicity

Short-term and subchronic exposure decreased body weight, weight gain, and feed consumption in rats and mice. Hematological effects also were observed. In male C57BL/6-J (WT), TNFR1-KO, and TNFR2-KO mice fed a diet containing DON (10 ppm [34 μmol/kg]) for 12 weeks, feed intake and body weight gain were significantly decreased compared to controls. By the end of the treatment period, overall weight gains were suppressed by 53, 64, and 67% in TNFR1, TNFR2, and WT groups, respectively. In another study, C57/BL6 mice were orally administered DON (0.014, 0.071, 0.355, 1.774, or 8.870 mg/kg [0.047, 0.24, 1.20, 5.987, or 29.93 μmol/kg] bw; equivalent to 0.05, 0.2, 1, 5, or 25 ppm, respectively) in a gum suspension three days per week for four weeks. Body weight gain was not affected, but DON significantly reduced spleen weight and increased liver weight at the high dose. DON also increased sodium, phosphate, creatinin, calcium, and total protein levels. Conflicting results were observed for the effect of DON on organ weights and organ-body weight ratios for other studies.

Male Wistar rats subcutaneously (s.c.) injected with DON (1 mg/kg [3 μmol/kg] bw) for three days and sacrificed 24 hours after the last treatment exhibited increases in blood insulin, glucose, and free fatty acids. In addition, a statistically significant increase in glycogen amount and a statistically significant reduction in triglycerides content in the muscle were observed. Similar effects were observed via i.p. infusion.

Chronic Toxicity

Mean daily food consumption by female $B6C7F_1$ mice (7 weeks old) fed experimental diets containing DON (20 mg/kg [67 μmol/kg]) for 16 weeks. DON reduced (3.6 ± 0.48 g vs. 2.94 ± 0.66 g) and mean body weight gain (12.94 ± 1.68 g vs. 2.76 ± 0.84 g). Serum immunoglobulin A (IgA) levels increased starting 8 weeks after initiation of DON-containing diet and serum IgA immune complex (IgA-IC) levels and mesangial IgA deposition started increasing 16 weeks after diet initiation. DON consumption also increased *ex vivo* IgA secretion from the spleen and Peyer's patch.

Synergistic/Antagonistic Effects

In *Salmonella typhimurium* strain TA98, the combination of DON and aflatoxin B1 (AFB1) had a greater mutagenic effect than AFB1 alone. In primary rat hepatocytes, the combination induced DNA synthesis at the S phase of the cell cycle. In a yeast (*Kluyveromyces marxianus*) bioassay, DON plus nivalenol (NIV) had a synergistic response on the toxicity, while DON plus T-2 toxin had an antagonistic effect. In mouse fibroblast L929 cells, a mixture containing DON, NIV, T-2 toxin, zearalenone, and fumonisin B1 produced greater inhibition of DNA synthesis than with treatment of each mycotoxin alone. Additionally, a synergistic interaction between DON and NIV was seen.

A synergistic carcinogenic effect was observed in NIH mice when Sterigmatocystin and DON were administered together. The number of animals with lung adenocarcinomas and glandular stomach dysplasia increased. In $B6C3F_1$ mice, coadministration of lipopolysaccharide (LPS) and DON significantly upregulated splenic interleukin (IL)-1β mRNA and plasma IL-1β protein expression compared to treatment with the toxins alone. DON-induced mitogen-activated protein kinase (MAPK) phosphorylation and IL-6 expression, however, were found to be suppressed by fish oil in the mice. Additionally, fish oil attenuated DON-induced immunoglobulin A (IgA) nephropathy in the animals. Fish oil constituents decosahexaenoic acid and eicosapentaenoic acid also suppressed the effect.

Cytotoxicity

In mouse thymocytes *in vivo*, DON (0.5-8.0 mg/kg [2-27 µmol/kg]) dose-dependently induced significant increases in apoptosis rates compared to controls. In addition, DON (4 and 8 mg/kg [13-27 µmol/kg]) significantly decreased the proliferation indexes of the treated cells *in vim*. In Swiss mouse 3T3 fibroblasts, 24-hour incubation with DON (78-2500 ng/mL [0.2-8.4 µM]) resulted in an $IC_{50}$ value (concentration inhibiting the incorporation of BrdU in DNA by 50%) of ~444 ng/mL.

Numerous studies have reported cytotoxic effects of DON in a variety of cell lines including pre-B lymphocyte REH, human pre-T lymphocyte Jurkat, hamster kidney-derived BHK21, mouse hepatoma MH-22a, HEp-2, CHO-K1, renal proximal tubule epithelial, normal human lung fibroblast, human dendritic, Caco-2, lung A549, monocytic U937, Hep-G2, MRC-5, human erythroleukemia K562, and human peripheral blood mononuclear cells. A dose-dependent increase in cell death was seen in rat liver clone-9 cells treated with DON (up to 100 µg/mL) along with a decrease in cellular double-stranded DNA content. Hepatocyte necrosis was observed in 75% (3/4) of rats 3 hours after injection of DON (10 mg/kg bw).

Reproductive and Teratological Effects

In three-month-old nulliparous female NMRI mice, i.p. injection of DON (3.3, 4.2, 5, or 10 mg/kg [11, 14, 17, or 34 µmol/kg] on gestation days 7 and 9 or 1.6, 2.5, or 3 mg/kg [5.4, 8.4. or 10 µmol/kg] daily on gestation days 7-10) produced high maternal deaths at the two higher doses. In embryos, the number of resorptions was dose-dependently increased in treated animals compared to controls. Skeletal abnormalities were observed. Exencephaly was mainly seen at 75 or 100 µg/30 g during the four-day treatment. At the higher dose and shorter exposure period, neural arch defects or fusion were mostly seen. In both experiments, vertebral bodies showed various deformities, hemivertebrae (except with 75 µg/30 g given for four days), and fused, branched, and/or cervical ribs.

DON (0.5, 1, 2.5, or 5 mg/kg [2, 3, 8, or 17 µmol/kg]) given to Sprague-Dawley female rats via oral gavage on gestation days 6-19 decreased mean gravid uterine weight at the highest dose. Average fetal body weight, crown-rump length, and ossification of fetal vertebrae also were significantly decreased at 2.5 and 5 mg/kg bw and 52% of litters (12/23) in the high-dose group were resorbed. Epididymal (right and left) and seminal vesicle weights were significantly reduced at the two highest doses in male Sprague-Dawley rats treated daily with DON (0.5, 1, 2.5, or 5 mg/kg [2, 3, 8, or 17 µmol/kg] bw) via gastric intubation for 28 days. Decreased prostate weight, spermatid numbers, and cauda epididymal sperm numbers, and increased sperm tail abnormalities (broken tails) also were observed at the high dose. Serum concentrations of follicle-stimulating hormone, luteinizing hormone, and testosterone were modulated in a dose-dependent manner. Increases in germ cell degeneration, sperm retention and abnormal nuclear morphology were observed at doses >2.5 mg/kg bw.

Carcinogenicity

The International Agency for Research on Cancer (IARC) concluded in 1993 that "[t]here is inadequate evidence in experimental animals for the carcinogenicity of deoxynivalenol." Overall, it was placed in Group 3, "not classifiable as to its carcinogenicity to humans." Subsequently, a two-year carcinogenicity study in mice was published. Dietary administration of DON did not result in an increased incidence of neoplasms in males or females; in males, there was a decreased incidence of liver neoplasms, probably a result of lower body weights. More recently, gastric intubation of NIH mice with DON (1.5 µg/kg [5.1 nmol/kg] bw) three times a week for 24 weeks was reported to have induced lung adenocarcinoma (37.5% of mice) and dysplasia of glandular stomach (25.0% of mice).

Genotoxicity

DON (1-10 µmole) increased the number of damaged Vero cells (cell with long tails, tail DNA, tail length, and tail motion) *in vitro* in a dose- and time-dependent manner. Short-term incubations (4 hours)

mainly induced an increase of the number of DNA fragments while longer inubation time (16 hours) mainly caused small size DNA fragments. DON also increased mean tail moment in Caco-2 cells in a dose-dependent manner at a concentration range (0.01-0.05 μM [3-15 ng/mL]) where apoptosis was not observed. Dividing cells had a greater sensitivity to DON than differentiated cells. In Chinese hamster V79 cells, DON fractions from samples of wheat (30 ng/mL [0.10 μM]), barley (200 ng/mL [0.675 μM]), and corn (300 ng/mL [1.01 μM]) induced chromosome aberrations.

Immunotoxicity

*In vivo* studies showed that DON induced MAPK phosphorylation and cytokine mRNA expression, as well as serum IgA and IgE levels. *In vivo* and *in vitro* studies show that it also superinduced proinflammatory cytokine gene expression. In TNFR1-KO mice, serum IgA and IgA-IC, and kidney IgA deposition were markedly decreased compared to WT and TNFR2-KO groups when given diets containing DON. Immunization of mice with cholera toxin had no effect on total serum IgA. DON induced cyclooxygenase-2 (COX-2) gene expression *in vivo* and *in vitro*. It inhibited nuclear protein binding to NRE-A, an IL-2 promoter negative regulatory element, in murine lymphoma EL-4 T cells and induced cytotoxicity and apoptosis in WEHI-231 B cells.

Other Data

In adipocytes isolated from male Wistar rats, DON (20 μM [5.9 μg/mL]) slightly stimulated basal lipogenesis but had no effects on insulin-induced lipid synthesis and lipolysis. DON also did not affect cell viability. In RAW 264.7 macrophages, PP1 (Src-family-tyrosine kinase inhibitor selective for Hck) and 2-AP (2-aminopurine) additively inhibited DON-induced caspase-3 activity and apoptosis, p53-binding activity, and MAPK phosphorylation. PFT (p53 inhibitor) canceled DON-induced caspase-3 activity and apoptosis, while SB 203580 (p38 inhibitor) canceled DON-induced p21 phosphorylation and p53 binding activity. In another study using macrophages, PD98059 (MEK1/2 inhibitor that inhibits ERK activation) and SB 203580 significantly reduced DON-induced prostaglandin E2 (PGE2) production.

Recent studies have shown that DON-induced inhibition of cellular proliferation may occur through arrest of the cell cycle at G2/M and increased expression of cyclin-related proteins.

DON stimulated *in vitro* estradiol, but not progesterone synthesis, in porcine granulosa cells at concentrations <0.03 μM [9 ng/mL]. In combination with T2, DON biphasically modulated progesterone synthesis. At a concentration of 0.01 μM [3 ng/mL], DON increased T2-toxin-induced stimulation of progesterone synthesis observed at a low concentration (0.0006 μM [0.2 ng/mL]) and inhibited its effects reported at a high concentration (0.002 μM [0.6 ng/mL]).

DON modulated hepatic biotransformation enzymes in mice subchronically exposed to DON by oral administration (0.014-1.774 mg/kg bw [0.047-5.987 μmol/kg]). DON also increased expression of P4502b and cytosolic glutathione *S*-transferase π and α subfamilies.

**Structure-Activity Relationships**

Nivalenol (NIV) [CAS No. 23282-20-4]

Like DON, NIV belongs to the type B-trichothecenes. It is one of the least acutely toxic trichothecenes; an oral $LD_{50}$ value of 39 mg/kg was reported for mice. The absorption of NIV is rapid and excretion is mainly via feces. The major effects of subchronic/short-term and chronic toxicity experiments in mice were reduced body weight gain, reduced feed efficiency, changes in organ weight, and hematotoxicity. NIV was embryotoxic and fetotoxic but not teratogenic in the animals. A two-year feeding study in female mice did not produce tumors. The IARC concluded there "there is inadequate evidence in experimental animals for the carcinogenicity of nivalenol" and put it in Group 3. NIV weakly induced

chromosomal aberrations in mammalian cells *in vitro*. It can be immunosuppressive and immunostimulatory.

Fusarenon X (FusX) [CAS No. 23255-69-8]
FusX is rapidly absorbed and excreted and deacetylated to NIV. The oral $LD_{50}$ values were 4.5 and 4.4 mg/kg bw in mice and rats, respectively. In short-term/subchronic and chronic studies, intrahepatic bile duct hyperplasia, atypical hyperplasia in the gastric and intestinal mucosa, and hypoplasia and atrophy of bone marrow, thymus, and spleen were reported. FusX was embryotoxic and fetotoxic but not teratogenic in mice. The IARC concluded there "there is inadequate evidence in experimental animals for the carcinogenicity of fusarenon X" and put it in Group 3. It produced some chromosome breaks in mammalian cells. Furthermore, FusX is immunosuppressive.

3-Acetyldeoxynivalenol (3-Ac-DON) [CAS No. 50722-38-8]
3-Ac-DON is deacetylated to DON by rabbit liver carboxy esterases. $LD_{50}$ values in mice indicate it to be acutely toxic. The main effects of an acute toxicity study with 3-Ac-DON in mice were on dividing cells of the duodenal crypts, thymus, and spleen. In short-term/subchronic studies in mice, feed consumption and body weights were reduced during the first few weeks of treatment. Liver weight was increased in a 48-day feeding trial with 3-Ac-DON. 3-Ac-DON was not mutagenic in the Ames test but did induce chromosomal aberrations in Chinese hamster V79 cells *in vitro*. It is immunosuppressive and immunostimulating.

15-Acetyldeoxynivalenol (15-Ac-DON) [CAS No. 88337-96-6]
15-Ac-DON is acutely toxic, with oral and i.p. $LD_{50}$ values of 34 and 113 mg/kg bw, respectively, in mice. It mainly affects the dividing cells of bone marrow, thymus, spleen, and intestines. In short-term/subchronic studies, feed consumption and body weight gain were reduced in mice. Additionally, absolute liver, kidney, and spleen weights were decreased, while relative spleen and kidney weights were increased. 15-Ac-DON is immunosuppressive and immunostimulating.

**Comparative Toxicity with Other Trichothecenes and Related *Fusarium* Mycotoxins**
DON and NIV are type B trichothecenes that often occur together in grain and feed sources; however, type A trichothecenes such as T-2 toxin or diacetoxyscirpenol or other *Fusarium*-produced mycotoxins (e.g., zearalenone, fumonisin B1, or fusarin C) may also occur in combination with DON and/or NIV. In general, type A trichothecenes tend to be produced in lower quantities than the type B but are more toxic. DON has been reported to be the least toxic type B and T-2 toxin the most toxic type A. All of the mycotoxins except fusarin C caused reproductive or developmental effects in mice as well as cytogenetic damage in mammalian cells *in vitro*. Both type A and B trichothecenes were found to be immunotoxic.

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## 1.0 Basis for Nomination

Deoxynivalenol (DON), a trichothecene mycotoxin produced by certain *Fusarium* species that frequently occur in corn, wheat, barley, rice, and other grains in the field, was nominated by the National Institute of Environmental Health Sciences for chronic toxicity and carcinogenicity studies and reproductive toxicity studies. The toxicological profile of DON is well described yet definitive long-term studies are generally lacking. In a previous two-year carcinogenicity study in mice, dietary administration of DON did not result in an increased incidence of neoplasms in males or females; in males, there was a decreased incidence of liver neoplasms, probably a result of lower body weights (Iverson et al., 1995 [PMID:8732880]). DON is genotoxic, immunosuppressive, teratogenic, and affects multiple reproductive endpoints. The widespread contamination of human foods and demonstrated toxicological activity of DON indicate the need for further studies to evaluate potential carcinogenicity and reproductive effects. Such definitive studies will also serve as index studies necessary for developing toxic equivalency factors (TEFs) for other tricothecene mycotoxins. Similar conclusions regarding the need for DON toxicological data have previously been articulated by several groups, including the Joint FAO/WHO Expert Committee on Food Additives and the European Commission Scientific Committee on Food (JECFA) (http://europa.eu.int/comm/food/fs/sc/scf/out44_en.pdf), and in a workshop organised by the ILSI Europe Natural Toxin Task Force (Larsen et al., 2004 [PMID:15342076]).

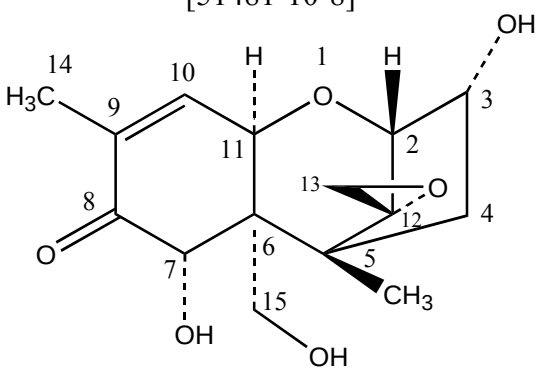
Recommendations of the JECFA

The following toxicological research needs were identified by the JECFA in their safety evaluation of DON (JECFA, 2001d):

- The results of comparative studies of toxicity and toxicokinetics would help to clarify species differences in sensitivity to DON.
- Studies are needed on the combined effects of DON and other trichothecenes that may be present in human food. As the trichothecenes have similar toxic properties, albeit with different potencies, the Committee recommended that toxic equivalency factors be developed for the trichothecenes, if sufficient data become available. Since DON is the most extensively studied trichothecene, the Committee further recommended that toxic equivalency factors be established relative to DON.
- In view of the widespread human exposure to DON, further studies on the genotoxicity of DON should be conducted, as well as a study of carcinogenicity in a second species (rat).

## 2.0 Introduction

Deoxynivalenol
[51481-10-8]



DON is a mycotoxin primarily produced by the fungus *Fusarium graminearum* (sexual stage, *Gibberella zeae*) (Cote et al., 1984 [PMID:6230342]). The other *Fusarium* species from which DON is isolated are *F. culmorum*, *F. roseum*, and *F. sporotrichioides* (Budavari, 1996; Lawlor and Lynch, 2001). The genus *Fusarium* is widely found in nature with various species growing as saprophytes on decaying vegetation and as parasites on various plants in the field or on harvested crops during storage (Cote et al., 1984 [PMID:6230342]). DON is a toxic byproduct of *Fusarium* head blight (FHB) which can make wheat unfit for milling and barley unsuitable for malting (Donley, 2004; USWBSI 2004).

## 2.1 Chemical Identification and Analysis

DON ($C_{15}H_{20}O_6$; mol. wt. = 296.32) is also called:

Dehydronivalenol
4-Deoxynivalenol
4-Desoxynivalenol
NSC 269144
Spiro[2,5-methano-1-benzoxepin-10,2′-oxirane], trichothec-9-en-8-one derivative
Trichothec-9-en-8-one, 12,13-epoxy-3,7,15-trihydroxy-(3α,7α)- (9CI)
Vomitoxin

Sources: Budavari (1996); Registry (2004)

The general methods for the quantitative determination of DON are thin-layer chromatography (TLC) and enzyme-linked immunosorbent assay (ELISA). For the simultaneous determination of several trichothecenes, gas chromatography (GC) is used with mass spectrometry (MS), tandem MS, electron capture detection (ECD), or flame ionization detection (JECFA, 2001a, 2002). Additionally, TLC can be used for the simultaneous determination of *Fusarium* mycotoxins (DON, diacetoxyscirpenol, and T-2 toxin) in maize-based foods. Recovery for DON added to various food samples was ~75% (Anaya et al., 2004). TLC (method 986.17) and GC (method 986.18) are the current Association of Official Analytical Chemists official methods for analyzing DON in whole wheat flour, white flour and bran (Codex Alimentarius Commission, 2007).

DON can also be detected by high-performance liquid chromatography (HPLC) in foods and cereals (JECFA, 2001a, 2002). The limits of detection and quantification for HPLC with ultraviolet (UV) detection were 10 and 50 µg/kg, respectively, for the analysis of DON in cereal samples (Czerwiecki and Wilcszynska, 2003). In combination with atmospheric pressure chemical ionization mass spectrometry, B-trichothecenes and the major metabolites of DON (nivalanol [NIV], 3-acetyl-DON [3-Ac-DON], 15-acetyl-DON [15-Ac-DON], de-epoxy-DON, and fusarenon X [FusX]) can be simultaneously determined (Razzazi-Fazeli et al., 2003 [PMID:14552813]).

Liquid chromatography (LC) allows the analysis of DON and other mycotoxins in biological matrices such as fungal liquid cultures, corn grain, insect larvae, and pig serum. Combined with MS, DON levels as low as 8 ppb were detected (Bily et al., 2004 [PMID:15008354]). Using reversed-phase LC with diode array detection, a 92-97% recovery of DON from spiked whole wheat flour samples was obtained. For levels <1 ppm, determination was by GC-ECD, and

DON recovery from spiked whole wheat flour samples was 91-103% (Walker and Meier, 1998 [PMID:9680699]). GC-MS was used to investigate trichothecene content in duplicate diets of children. DON was detected in all 74 samples analyzed at a mean concentration of 5.8 µg/kg; the limits of detection and quantification were 0.10 and 0.30 µg/kg, respectively (Schothorst et al., 2005 [PMID:15895611]). The purity of crystalline DON from two commercial sources was determined by LC with a variable wavelength detector, LC/MS/MS, GC-ECD, and UV spectrophotometry. The purities of DON from Sigma and Biopure were calculated at >96 and >98%, respectively (Krska et al., 2004 [PMID:15295885]).

Sequence characterized amplified region (SCAR) primers have been used to identify *Fusarium* species causing wheat scab. The three *Fusarium* species (*F. graminearum*, *F. culmorum*, and *F. avenaceum*) were identified using polymerase chain reaction assay in sporodochia collected from infected wheat spikelets. The SCAR method confirmed the preliminary identification of the species using microscopic identification of macroconidia under light microscope. DON levels ranged from 0.01-15.32 µg/g in kernels of the ears samples and were >1 µg/g in 5/25 samples tested (Chekowski et al., 2002). Fluorescence polarization can also serve as a tool for the determination of deoxynivalenol in wheat (Maragos et al., 2002 [PMID:11962698]).

An acoustic screening method ([bio-] sensor-based techniques and non-invasive methods based on infrared [IR] and other techniques) can be successfully used for an effective control and screening of cereals contaminated with *Fusarium* species (Juodeikiene et al., 2004). In maize samples, a rapid screening method, Fourier transform mid-IR spectroscopy with attenuated total reflection, was applied to detect the presence of *F. graminearum.* DON was determined by GC-ECD and identified in 75% of the samples with concentrations in the tested samples ranging from 0.13-2.59 mg/kg (Kos et al., 2002).

Under the United States Grain Standards Act, the Grain Inspection, Packers and Stockyards Administration (GIPSA) can provide DON testing service for wheat, barley, oats, and corn. Their approved test kits use either fluorescence ELISA technology (e.g., Neogen-Veratox, Romer-AccuTox, and Diagnostix-EZ Quant DON) or HPLC analysis to assess DON for Board Appeal inspections (USDA GIPSA, 2002).

An array biosensor was used to detect and quantify DON in spiked-food and air samples. Extracts (using methanol-water) of the food samples (cornmeal, cornflakes, wheat, barley, and oats) were assayed without cleanup or preconcentration. The limits of detection for the food samples ranged from 1-50 ng/g and for the DON-spiked air samples was 4 ng/mL (Ngundi et al., 2006 [PMID:16646473]).

## 2.2 Physical-Chemical Properties

| Property | Information | Reference(s) |
|---|---|---|
| Physical State | colorless fine needles | Budavari (1996); JECFA (2001a) |
| Boiling Point (°C) | 543.9±50.0 | Registry (2004)* |
| Melting Point (°C) | 151-153 | Budavari (1996) |
| Flash Point (°C) | 206.9±42.5 | Registry (2004)* |
| Vapor Pressure (Torr) | $4.26 \times 10^{-14}$ @ 25 °C | Registry (2004)* |
| Soluble in: | polar organic solvents (e.g., aqueous methanol, ethanol, chloroform, acetonitrile, and ethyl acetate) and water | Gimeno (undated); JECFA (2001a) |
| Molar Solubility | ≥0.1 - <1 M @ pH 1-10 | Registry (2004)* |
| Bioconcentration Factor | 1 @ pH 1-10 | Registry (2004)* |
| Adsorption Coefficient ($K_{oc}$) | 3.79 @ pH1-10 | Registry (2004)* |
| Octanol-water partition coefficient (log P) | -1.468±0.535 | Registry (2004)* |

*calculated using Advanced Chemistry Development (ACD/Labs) Software Solaris V4.67 (© 1994-2004 ACD/Labs)

DON, stable at a temperature of 120 ºC, is not decomposed under mild acidic conditions. Its three hydroxyl groups can be derivatized (e.g., esterified) (JECFA, 2001a). DON is optically active, has great stability during storage/milling and in the processing and cooking of food, and does not degrade at high temperatures (Gimeno, undated). The optimal temperature range for DON mold is 21-29 °C with moisture levels >20%. The fungus has two growth cycles, with the mold growing during the warm daytime temperatures and the toxins developing during the cool nighttime temperatures (USDA GIPSA, undated).

## 2.3 Commercial Availability

For experimental purposes, DON can be purchased from Sigma (St. Louis, MO) (e.g., Berek et al., 2001 [PMID:11259866]; Tajima et al., 2002 [PMID:11955675]; Uzarski and Pestka, 2003 [PMID:14710595]).

## 3.0 Production Processes

No data were available.

## 4.0 Production and Import Volumes

No data were available.

## 5.0 Uses

DON is considered a marker for other toxins in grains and feeds (Lawlor and Lynch, 2001; North Dakota State University, 2000).

## 6.0 Environmental Occurrence and Persistence

The occurrence of DON is mainly linked with *F. graminearum* and *F. culmorum*. The former species grows optimally at 25 ºC while the latter at 21 °C. A summary of data reported for DON concentrations in food commodities in several countries (e.g., Brazil, Canada, Germany, Sweden, the U.K., and the U.S.) as provided in the literature produced the following mean concentration ranges for commodities collected from 1979 to 2000: 4-760 µg/kg for oats, 4-9000 µg/kg for barley [high mean was for U.S. malting barley grown in 1993], 1-5700 µg/kg for wheat, 13-240 µg/kg for rye, 3-3700 µg/kg for maize, and 6-5100 µg/kg for rice. The incidence of

contaminated samples ranged from 27% for rice to 68% for oats (JECFA, 2001a). The incidence and concentration of DON in grains from contries around the world, excluding consumer products, reported in the literature and privileged communications (1998-2006) for the surveyed period of 1992-2005 as presented in a second authoritative review for corn (maize), wheat, and barley were somewhat different. Maximum DON contaminations were often less than the high end of the mean ranges shown above and the means were generally less than 850 µg/kg (highest mean was 2919 µg/kg for corn silage). The incidence of positive samples was up to 100%. Wide variations in concentrations and incidences seen from year to year were usually attributed to different climatic conditions. In one study, high DON concentrations were attributed to damp climate, cool conditions, and delayed harvest times. Maize was usually the grain most contaminated with *Fusarium* mycotoxins (Codex Alimentarius Commission, 2007).

Monoacetylated DON-derivatives were reported along with DON in a few studies; they often occur at 10-20% of the DON content. In one study, 26% of Italian maize samples contained 15-Ac-DON at concentrations up to 3500 µg/kg. Breads on the German market in 1999 had an 8% incidence of 3-deoxynivalenol (Codex Alimentarius Commission, 2007). In North America, the *F. graminearum* chemotype that produces 3-Ac-DON has higher fecundity and growth rates and in western Canada, this chemotype increased 14-fold from 1998 to 2004 (Ward et al., 2008 [PMID:18035565]). The predominant *F. graminearum* in North America has had the 15-Ac-DON chemotype (Starkey et al., 2007 [PMID:17451976]).

Three less toxic thermal degradation products of DON known as nor-DONs A, B, and C have been determined in thermally processed foods on the German market with incidences of 62, 35, and 29%, respectively, when DON incidence was 94%. Concentrations of DON in the positive samples were 10-301 µg/kg while concentrations of the metabolites were 1-36 µg/kg (Bretz et al., 2006 [PMID:16910743]).

DON-3-glucoside is a recently discovered "masked" mycotoxin potentially present in wheat and maize that is not detected by the usual analytical methods, but which may release DON during hydrolysis. It was found in five of five naturally contaminated wheat samples at concentrations that were 4-12% of DON concentrations (Berthiller et al., 2005 [PMID:15853382]). DON-3-glucoside concentrations exceeded free DON concentrations in final beers produced from DON-contaminated barley malt (Lancova et al., 2008 [PMID:18484301]).

DON levels in wheat grain samples harvested by hand were more representative of levels in the field than samples harvested by machine. The largest factor associated with variation in DON levels was the year (48%), followed by cultivar (27%), and the crop grown one year previous to wheat (14-28%). Agronomic factors (tillage system, crops planted three years before wheat, or type of nitrogen fertilizer applied in the spring), however, had no effect on DON levels (Schaafsma et al., 2001).

A few studies have reported traces of DON in environmental media. Mean DON concentration in Swiss rivers in July and August 2007 was 22 ng/L. Drainage water collected in June and July from fields growing winter wheat experimentally infected by *Fusarium graminearum* contained DON at 23 ng/L to 4.9 µg/L (Bucheli et al., 2008 [PMID:18197623]). Wastewaters from food production and bioethanol production may also contribute to DON contamination of surface

water (Bucheli et al., 2005; Hanschmann and Krieg, 2006). DON was not detected in swab or tape-lift samples from surfaces of U.S. outdoor recreational environments (Sudakin and Fallah, 2008 [PMID:18615277]) and it was rarely reported as a contaminant of indoor air due to mold-infested building materials (Jarvis and Miller, 2005 [PMID:15565335]).

Potential Exposures in the USA

Epidemics of FHB, a major disease in U.S. and Canadian wheat and barley, have been increasing since 1993 (Alexander, 2008). Genetically modified crops such as wheat and corn have shown reduced toxicity of the trichothecenes produced or increased resistance to infection (e.g., Alexander, 2008; Hammond et al., 2004 [PMID:14995151]). A combination of management strategies (variety resistance, fungicides, and/or crop rotation) has proved effective in reducing DON concentrations and disease severity in wheat and barley production in the U.S. (McMullen et al., 2008). However, increasing imports of wheat, corn, barley, oats, rye, and rice provide additional contributions of DON and other *Fusarium* toxins to U.S. processed foods (Ryu and Bullerman, 2008 abstr.).

In 1982, wheat samples naturally infected by *F. graminearum* Schwabe that were collected from mills in Oklahoma, Missouri, Kansas, and Minnesota and from fields in Nebraska and Kansas, contained DON concentrations ranging from 21.3 ppm [71.9 µmol/kg] (Nebraska lot 1, bran) down to 1.1 ppm [3.7 µmol/kg] (Missouri lot 2, break flour); most samples exceeded the Food and Drug Administration (FDA) suggested concentration of 2 ppm [7 µmol/kg]. DON was not removed by cleaning or milling nor destroyed by baking bread and was distributed throughout all fractions of the milled wheat (Abbas et al., 1985 [PMID:4051489]).

Resistance to mycotoxin contamination was compared in field samples harvested from 45 commercial corn hybrids in Mississippi and five single-cross aflatoxin-resistant germplasm lines during years with high and moderate heat stress. In high heat stress, DON (0-1.5 mg/kg [6.1 µmol/kg]) was detected in one-fourth of the samples (Abbas et al., 1985 [PMID:4051489]). Pennsylvania corn silage samples collected in 2001 and 2002 at harvest and after fermentation were plated on selective media and fungi identified by morphology and DNA sequence data. DON was the most prevalent toxin with increased levels in 2002; drought conditions were suggested as the responsible factor (Nagy and Kuldau, 2003). Occurrence of DON in corn silage, corn grain, and all feed samples submitted by producers in North Carolina over a nine-year period were 66% (mean±sd, 1991±2878 ppb), 70% (1504±2550 ppb), and 58% (1739±10880 ppb), respectively (Whitlow and Hagler, 2002).

Effects of cleaning, milling, and pasta processing on microbial loads and DON concentrations were determined on the 2001 durum wheat crop grown in the Northern Plains. Aerobic plate counts (APCs), mold and yeast counts (MYCs), internal mold infection, and internal *Fusarium* infection (IFI) were lowest in grain samples from Montana (not detected) and highest in grain from northwestern North Dakota (23 µg/g [78 µmol/kg]). DON positively correlated with APCs, MYCs, IFI, damaged kernels, total defects, U.S. grade number, and tombstone kernel content and negatively correlated with test weight, vitreous kernel content, and kernel weight. APCs, MYCs, and DON concentrations were lower in semolina versus whole grain. Processing semolina into spaghetti did not change DON concentrations; microbial loads in spaghetti were all within specifications (Manthey et al., 2004 [PMID:15083730]).

Sixty-six isolates of *F. graminearum* associated with FHB were collected in North Carolina and tested for *in vitro* growth rate, DON production, and pathogenicity on three cultivars of soft red winter wheat. Randomly amplified polymorphic DNA analysis revealed high levels of genotypic diversity among isolates. *In vivo* levels of DON measured for five isolates associated with the highest levels of disease and the five isolates associated with the lowest level of disease showed no significant differences. All ten isolates produced detectable levels of DON *in vivo*; mean disease ratings ranged from 3.4-96.4%. *In vitro* DON levels ranged from 0-7176.2 ppm [24.218 µmol/kg]. Analysis of phenotype and genotype among isolates demonstrated diversity in a single plot, in a single location, and in North Carolina. A variable pathogen population of *F. graminearum* exists in North Carolina and members of this population can be both highly pathogenic on wheat and produce high levels of detrimental toxins, indicating a potential threat for problems with FHB in this state (Walker et al., 2001).

Test samples (n=181) from ten barges of shelled corn were divided into fines and clean components. DON concentration in the fines and clean component samples averaged 689.0 and 206.1 ng/g [2.325 and 0.6955 nmol/g], respectively (Whitaker et al., 2003 [PMID:1497901]).

Results from two U.S. surveys of data from grain samples collected from 1994-2003 reported that 59% of wheat samples (2524) had DON concentrations >500 µg/kg; 18.6% had >500-1000 µg/kg, 39.8%, >1000-6000 µg/kg, and 0.6% >6000 µg/kg. For barley samples (2106), 62% had concentrations ≥490 µg/kg; 14.5% had 490-990 µg/kg; 28.5%, >990-4990; and 18.6%, 4990 to >5000 µg/kg. Wheat-based products (728) that included bran, flour, and other milled products that were collected in the U.S. from 2000-2004 [cited in a 2005 privileged communication] contained DON concentrations >100 µg/kg in 31%, 37%, and 36%, respectively. The incidence of samples >1000 µg/kg was 17.5%, 1.0%, and 1.8%, respectively (Codex Alimentarius Commission, 2007).

Potential Exposures in Canada

Samples (n=363) of cereal-based infant foods (i.e., oat-, barley-, soy-, and rice-based formulas, mixed-grain, teething biscuits, and creamed corn) were collected from the Canadian retail marketplace for a period of three years and analyzed for targeted mycotoxins. Soy-based cereals (which usually contain corn) had a 100% incidence of DON, which also was detected in 63% of all of the cereal-based samples analyzed (Lombaert et al., 2003 [PMID:12775469]).

DON concentrations in maize, wheat, barley, and oat samples collected in eastern Canada from 1991 to 1998 were 8.9, 31.3, 22.4, and 1.4%, respectively (Campbell et al., 2002). DON levels of ≥0.05 ppm [≥0.17 µmol/kg] were found in grain samples from 7/10 Manitoba and 1/19 Saskatchewan crop districts in 1996 and from 8/11 Manitoba and 1/20 Saskatchewan crop districts in 1997 (Clear et al., 2000a). DON levels ≥0.10 ppm [≥0.34 µmol/kg] (maximum 0.34 ppm [1.1 µmol/kg]) were found in oat seed (*Avena sativa*) from two Manitoba crop districts in 1996 and three in 1997. DON was also detected in composite seed samples from three Sasakatchewan and two Alberta crop districts (Clear et al., 2000b).

Commercial lots of two hard red spring wheats (wheat 1 and 2) from the 1981 Quebec wheat crops contaminated with DON at 7.5 µg/g [25 µmol/kg] and 1.4 µg/g [4.7 µmol/kg] were

subjected to cleaning, tempering, and scouring processes and then prepared for milling experiments in the Grain Research Laboratory (pilot mill), Allis-Chalmers mill (experimental mill), and Canadian International Grains Institute (commercial-scale pilot mill). Flours were baked into bread, cookies, and doughnuts. DON was distributed throughout the milling fractions with relatively high retention in all streams. A two-fold increase in DON concentrations was observed in shorts and feed flour fractions; there were fewer increases in bran from wheat 2. No DON losses occurred during cleaning, milling, or baking processes were found (Scott et al., 1984 [PMID:6537355]).

DON concentrations measured in wheat samples from 399 fields in southern Ontario from 1996 to 2000 ranged from undetectable to 29 µg/g [98 µmol/kg]. Weather variables (daily rainfall, daily minimum and maximum temperatures, and hourly relative humidity) affected the presence of DON in three critical periods: 4-7 days before heading (concentrations increased with the number of days >5 mm of rain and decreased with the number of days <10 $^{o}$C), 3-6 days after heading (concentrations increased with the number of days of rain >3 mm and decreased with days exceeding 32 $^{o}$C) and 7-10 days after heading (concentrations increased with the number of days with >3 mm of rain). No relationship between relative humidity and DON concentration was observed (Hooker et al., 2002).

Potential Exposures in Other Countries

Settled dust samples were collected during grain-handling and storage activities in 11 municipalities in climatically different gain producing districts in Norway. DON was detected in oat (n=32, median value = 23 µg/kg) and spring wheat (n=13, median value = 34 µg/kg) samples, but not barley samples (n=59). Studies indicated that DON concentration was correlated not only with cereal species, but also with fungal forecasts and rainfall (Nordby et al., 2004).

In samples collected from local markets in Egypt, DON was detected in five wheat samples (103-287 µg/kg [0.348-0.969 µmol/kg]), one flour sample (188 µg/kg [0.634 µmol/kg]), and one bread sample (179 µg/kg [0.604 µmol/kg]). A 4-kGy gamma-irradiation exposure decreased DON concentrations in flour (125 µg/kg [0.422 µmol/kg]) and wheat (85 µg/kg [0.29 µmol/kg]), while 6 kGy completely eliminated fungal flora in both samples. Gamma-irradiation also considerably reduced the natural occurrence of *Fusarium* mycotoxins in bread (<5 µg/kg [0.02 µmol/kg]) (Aziz et al., 1997 [PMID:9113669]). DON was also detected in white corn (28.8 µg/kg [10.3 µmol/kg]) and popcorn (10.1 µg/kg [0.0341 µmol/kg]) samples collected from various districts in Egypt (El-Sayed et al., 2003 [PMID:13678256]).

The incidence and levels of DON contamination were surveyed in Italian marketed foods. In 202 samples of raw materials and processed cereal foods (bread, pasta, breakfast cereals, biscuits, baby and infant food), 84% were contaminated with DON at 0.007-0.930 µg/g [0.024-3.14 µmol/kg]; the highest levels were detected in raw cereals and whole meal flours (Cirillo et al., 2003 [PMID:12881130]).

Samples of wheat (n=201), barley (n=106), and oats (n=13) from the 1999 U.K. harvest and a small number of organic samples were collected from farms, central stores, mills, maltings, and ports from February to April 2000. Results from a survey of these samples showed DON was

detected in 88% of all samples with 83% having concentrations below 100 µg/kg [0.337 µmol/kg]; the maximum level was 600 µg/kg [2.02 µmol/kg] (MacDonald et al., 2004 [PMID:14754640]).

In 1999, a total of 60 wheat flour samples collected from food stores and mills located in southwest Germany had concentrations of DON significantly higher for wheat flour produced by conventional (65-1379 µg/kg [0.22-4.654 µmol/kg]) than by organic agricultural methods (95-756 µg/kg [0.32-2.55 µmol/kg]) (Schollenberger et al., 2002 [PMID:11843417]). In an investigation of the occurrence of *Fusarium* infection and mycotoxin contamination in 2-15% of grains infested during 1995-1998 at three climatologically differing localities in Rhineland, Germany, the species frequently isolated were *F. avenaceum*, *F. poae*, *F. culmorum*, and *F. graminearum* with a mean DON content ranging from 19 µg/kg [0.064 µmol/kg] (in 1995) to 310 µg/kg [1.05 µmol/kg] (in 1998). Rates of infection and mycotoxin concentration were lower in organic farming than in conventional farming systems (Birzele et al., 2002).

A total of 84 samples of maize collected directly from fields or as grain from merchants in southern and central Moravia (Czech Republic) from 1998-1999 had DON concentrations of 25-285 µg/kg [0.084-0.962 µmol/kg] in 95.2% of the samples (Nedelnik, 2002).

Strains (n=129) of *Fusarium* species were obtained from 215 samples of soil and agricultural products in Korea and ten were identified as DON-producing strains. The DON concentration ranged from 20-90 µg/g [0.067-0.30 µmol/kg] in rice medium, with the corn-isolates having the maximum concentration (King et al., 2001).

## 7.0 Human Exposure

Potential human exposure to DON may occur from ingestion of foods derived from contaminated oats, corn, wheat, barley, rice, and other grains in the field. It has also been detected in buckwheat, popcorn, sorghum, triticale, and other food products for human consumption such as flour, bread, breakfast cereals, noodles, infant foods, pancakes, and malt and beer (JECFA, 2001a, 2002; Perkowski, 2000). (See also Section 6.0.) DON concentrations in top fermentation beers marketed in the Netherlands were 26 to 41 µg/L [0.088-0.14 µM] and in several German beers were >200 ng/mL [0.675 µM] (Schothorst and Jekel, 2003; Scott, 1996 [PMID:8757446]). Beer samples collected from European retail markets showed DON contamination ranging between 4.0 and 56.7 ng/mL [0.013 and 0.191 µM]. Drinking can contribute significantly to the tolerable intake of DON, especially for frequent beer consumers (Papadopoulou-Bouraoui et al., 2004 [PMID:15204540]). Eggs can also be a human exposure route for DON; however, the levels are insignificant compared to other sources. Laying hens fed a diet containing DON (~20 mg/kg [67 µmol/kg]) in wheat produced eggs contaminated with maximum levels of 10 mg/kg [34 µmol/kg] DON (Sypecka et al., 2004 [PMID:15315386]). Several studies reported negligible transfer of DON from the feed of dairy cows to their milk. In a recent study in Germany, DON was not detected in fresh milk from cows fed a diet containing DON and other *Fusarium* toxins but its metabolite deepoxy-DON was measured at 1-1.5 µg/kg (Keese et al., 2008 [PMID:18803258]). A simulation model that neglected DON metabolism predicted DON concentrations in milk of 1 µg/kg (Coffey et al., 2009).

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has estimated the dietary intake of DON in the GEMS/Food regional diets: 0.78 µg/kg [2.6 nmol/kg] body weight (bw)/day from the African diet, 1.2 µg/kg [4.0 nmol/kg] bw/day from the Latin American diet, 1.4 µg/kg [4.7 nmol/kg] bw/day from the European diet [note: The U.S. was grouped in this category], 1.6 µg/kg [5.4 nmol/kg] bw/day from the Far Eastern diet, and 2.4 µg/kg [8.1 nmol/kg] bw/day from the Middle Eastern diet. In Europe, Latin America, and the Middle East, the main source was wheat. A provisional maximum tolerable daily intake (PMTDI) of 1 µg/kg bw was established (JECFA, 2001a). In the Netherlands, 80% of one-year-old children exceeded the PMTDI, and 20% of them had twice the value. Although the major source of DON intake was bread, porridges were also a significant source for the children; the average DON level in wheat was 446 µg/kg [1.51 µmol/kg] (measured in samples during September 1998-January 2000) (Pieters et al., 2001). In a more recent study in the Netherlands, the diet of 74 children was reported to contain a mean of 5.8 µg DON/kg with nine of the diets exceeding the tolerable daily intake of 1 µg/kg bw (Schothorst et al., 2005 [PMID:15895611]). A mean dietary intake of DON in France was reported to be 451 ng/kg bw in children and 281 ng/kg bw in adults (Leblanc et al., 2005 [PMID:16019841]).

High concentrations of fungi were noted in both grain and grain-dust samples during the threshing of cereals by a combine harvester. DON concentrations ranged from 0.015-0.068 µg/g [0.051-0.23 µmol/kg] in wheat grain samples. A significant correlation was observed between the occurrence of fungi of *Fusarium* species and the concentration of pathologic mycotoxins, thus confirming a considerable occupational risk among farmers engaged in grain threshing due to inhalation of pathogenic species of filamentous fungi and mycotoxins (Krysinska-Traczyk et al., 2003 [PMID:12923995]).

Minimal human exposure to DON seems to occur from the consumption of meat from animals that have ingested DON from their feed. DON concentrations were measured in serum, bile, liver, kidney, musculus longissimus and back fat of pigs given diets containing 25 or 50% wheat contaminated with low concentrations of DON (2.5 mg/kg) in mash or pellet diets for 11 weeks. In muscle tissue, the maximum concentration detected in pigs fed a 25% wheat diet was 2.2 ng/g and in those fed a 50% wheat diet it was 5.2 ng/g. In the liver, the concentration from each diet was 3.6 and 4.8 ng/g, respectively, and in the kidneys, it was 17.5 and 19.3 ng/g, respectively. [The metabolite de-epoxy-DON was detected only in the kidneys (0-2.4 ng/g).] The maximum carry over factor from the diet (the sum of DON and de-epoxy-DON in tissues + fluids divided by the DON concentratiaon in the diet) were 0.0043 for muscle, 0.0064 for liver, and 0.0319 for kidney (Doll et al., 2008 [PMID:18465777]). These factors were similar to those found in another study in which pigs were fed a *Fusarium* toxin-contaminated diet containing 6.68 mg/kg DON for 12 weeks. The maximum carry over factors in this study were 0.0031, 0.0059, and 0.0193 in muscle, liver, and the kidneys, respectively. DON (0-8 µg/kg; mean 2.4 µg/kg) also was detected in back fat of the pigs (Goyarts et al., 2007 [PMID:17454110]). Overall, the contribution of DON from edible tissues of DON-exposed animals to human DON exposure was negligible.

Workers involved in grain handling or storage have potential inhalation exposure to DON. A recent German study measured blood plasma concentrations of mycotoxins in workers exposed

to the dust. DON concentrations in air and dust were 2 ng/m$^3$ and 416 ng/g, respectively (Mayer et al., 2007a,b).

## 8.0 Regulatory Status

In 1982 (and updated in 1993), the FDA established the following advisory guidelines for DON in food and feed: 1 ppm for finished wheat products (e.g., flour, bran, and germ) for human consumption; 10 ppm in grain and its byproducts for ruminating beef cattle, cattle in feedlots older than four months, and poultry (not to exceed 50% of diet); and 5 ppm in grain and its byproducts for swine (not to exceed 20% of diet) and for all other animals (not to exceed 40% of diet). No standard, however, has been established for raw grains intended for milling processes for human food products (FDA, 2008; McMullen and Stack, 1999; USDA GIPSA, 2002; Whitlow and Hagler, 2002). Discussion of proposals for maximum levels for rye and rye-derived products were on the agenda of the 36$^{th}$ Session of the Codex Committee on Food Additives and Contaminants (USDA FSIS, 2004). The session agreed to suspend consideration of the levels for DON and to request additional information regarding its occurrence in cereals, national guidelines or levels, methods of analysis, etc. (Codex Alimentarius Commission, 2007). The European Community supports the establishment of maximum levels for DON within Codex Alimentarius for raw cereal grains and all products derived from cereals and its grains for human consumption (EC, 2003).

The Agricultural Research Service (ARS) food safety national research program controls (the potential for) the presence of mycotoxins in crops, which includes DON in wheat and barley (USDA ARS, 2005). Under the FDA compliance program, milled wheat products (i.e., whole wheat flour, white flour, and bran) and samples of bran that may be used as a component of bran cereal are to be collected at wheat product and cereal manufacturers, respectively, for the analysis of DON (FDA, 2008).

Over 35 countires have established regulatory limits or guidelines for DON in foods and animal feed. The guideline levels for cereals and finished cereal products for humans ranged from 100-2000 µg/kg (depends on consumer age and stage of processing of the grain); levels in diets for swine, poultry, and cattle ranged from 500-10,000 µg/kg (Codex Alimentarius Commission, 2007). The European Union maximum level of DON for processed cereal-based baby foods and foods young children are 200 µg/kg (Verstraete, 2008).

## 9.0 Toxicological Data

### 9.1 General Toxicology

In 2001, the JECFA published a monograph summarizing the safety data of certain mycotoxins in food, including DON. In general, acute exposure to DON resulted in decreased feed consumption (anorexia) and vomiting (emesis). Short- or long-term exposure to DON caused reduced growth, with higher doses affecting the thymus, spleen, heart, and liver. Biochemical studies were conducted in rats, pigs, sheep, cattle, and chickens. DON was metabolized by de-epoxidation and glucuronidation to less toxic metabolites. In reproductive and developmental effects studied in mice, rats, rabbits, hens, and pigs, DON significantly decreased caudal epididymal weights in B6C3F$_1$ and IL-6KO mice. Additionally, it induced chromosomal aberrations *in vitro* and *in vivo* but failed to produce gene mutations *in vitro* in several assays (e.g., the Ames test and in Chinese hamster lung cells). This report makes no attempt to

duplicate the data found in the review; instead, the reader is referred to the JECFA monograph (JECFA, 2001a). Supplemental information for studies in laboratory animals (e.g., rats and mice) is presented in the following sections.

Recently the Codex Alimentarius Commission published its discussion paper on DON. The paper includes many of the studies cited in this current report and some that are not. As with the JECFA monograph, no attempt was made to duplicate all of the data from this paper and the reader is instead referred to the discussion paper itself (Codex Alimentarius Commission, 2007).

*[Note: Numerous recent studies utilized domestic animals, particularly pigs: e.g., reproductive/teratological effects - Alm et al., 2002 [PMID:12423645], Doll et al., 2003a [PMID:15595624]; ADME - Danicke et al., 2004 [PMID:15195910], Doll et al., 2003b, Goyarts and Danicke, 2006 [PMID:16326049], Zielonka et al., 2004; and clinical aspects - Lawlor and Lynch, 2001. (See also Section 14.0.)]*

### 9.1.1 Human Data

DON does not pose a threat to public health among the general population. In rare cases, acute temporary nausea and vomiting have been reported (USDA GIPSA, 2002). Other effects include diarrhea, abdominal pain, headache, dizziness, and fever; these are similar to the gastrointestinal conditions ascribed to microbes. Several epidemiological studies, mostly in Asia, have been conducted; the acute effects were attributed to consumption of *Fusarium*-contaminated grains and the presence of DON at 3-93 mg/kg [0.01-0.31 mmol/kg] (JECFA, 2001a). In 15 urine samples collected from female inhabitants of Linxian County, China, a high risk exposure region for DON and for esophageal cancer, and Gejiu, a low risk region, DON was detected in all samples; mean levels were 37 and 12 ng/mL [0.12 and 0.040 µM], respectively (Meky et al., 2003 [PMID:12480302]).

In one-year-old Dutch children exposed to DON levels above the PMTDI, reductions in body weight and relative liver weight were estimated at 2.2 and 2.7% (confidence interval: 0.2-25%), respectively (Pieters et al., 2001).

DON was detected in the urine of 296 of 300 subjects whose cereal intake was monitored and urinary DON concentrations were significantly associated with cereal intake ($p < 0.0005$). The geometric mean concentrations were 6.55, 9.63, and 13.24 µg DON/day for low-, medium-, and high-cereal intake groups, respectively. Consumption of other grain-based foods such as breads, cereals, and pasta also were significantly associated with urinary DON concentrations (Turner et al., 2008 [PMID:18197294]).

*In vitro* studies using human intestinal Caco-2 cells suggested that DON crosses the intestinal mucosa through a paracellular pathway, though contribution by passive transcellular diffusion could not be ruled out (Sergent et al., 2006 [PMID:16442754]).

### 9.1.2 Chemical Disposition, Metabolism, and Toxicokinetics

Male B6C3F$_1$ mice were orally administered DON (25 mg/kg [84 µmol/kg]) to assess the kinetics of DON distribution and clearance. DON was detectable in the plasma, liver, spleen, and brain from 5 minutes up to 24 hours postadministration. DON was detectable in the heart

and kidney from 5 minutes up to 8 hours postadministration. The highest plasma concentrations were detected from 5 to 15 minutes after dosing. The maximum DON concentrations in plasma 8 and 24 hours after dosing were 5% and 2%, respectively. Clearance followed two-compartment kinetics ($t(1/2)\alpha$ = 20.4 minutes, $t(1/2)\beta$ = 11.8 hours). DON concentrations (mg/kg) in other tissues 5 minutes after dosing were 19.5 ± 1.9 in liver, 7.6 ± 0.5 in kidney, 7.3 ± 0.8 in spleen, 6.8 ± 0.9 in heart, and 0.8 ± 0.1 in the brain (Pestka et al., 2008).

The effects of oral and intranasal exposure of adult female mice to DON (5 mg/kg [0.02 mmol/kg] bw) on tissue distribution was evaluated. Both exposure routes produced maximal DON concentrations in plasma, spleen, liver, lung, and kidney within 15-30 minutes. Levels declined by 75-90% after 2 hours. Plasma and tissue concentrations were 1.5-3 times higher after intranasal compared to oral exposure (Amuzie et al., 2008 [PMID:18433975]).

In male $B6C3F_1$ mice orally given DON (25 mg/kg [84 µmol/kg] bw), maximum levels of DON were detected in the following tissues at 30 minutes:

| Organs | Concentrations* (ng/g) |
|---|---|
| kidney | 5680 ± 1480 |
| heart | 4530 ± 1140 |
| plasma | 4430 ± 440 |
| liver | 3900 ± 206 |
| thymus | 3640 ± 105 |
| spleen | 2990 ± 110 |
| brain | 763 ± 61 |

*mean ± standard deviation

The concentrations were significantly higher in all the organs up to eight hours compared to controls (Yordanova et al., 2003 abstr.).

In male Sprague-Dawley rats administered a single dose of [$^{14}$C]DON (5 mg/kg [0.02 mmol/kg] bw) via gavage, the highest levels of radiolabeled DON (~291 ng/mL [0.982 µM]; 0.37% of dose) were detected in the plasma at eight hours. The binding of [$^{14}$C]DON to plasma proteins was ~9% of the total plasma DON. Of the administered dose, 37% was excreted in urine at 72 hours, and DON-glucuronide was the major urinary metabolite (Meky et al., 2003 [PMID:12480302]).

### 9.1.3 Acute Exposure

Five-week-old BALB/c female mice were orally gavaged with DON (10 mg/kg [34 µmol/kg] bw) or 30 µL saline (controls) followed by intranasal instillation of reovirus to assess the effects of DON on viral bronchopneumonia. Ten days after instillation of reovirus expression of the reovirus L2 gene was 10-fold higher in lungs of DON-treated mice compared to saline controls. DON suppressed interferon (IFN)-α, IFN-αβ receptor, and IFN-γ receptor gene expression and inhibited induction of double-stranded RNA-activated protein kinase R and oligoadenylate synthase 2 (type 1 INF-dependent antiviral genes). The mild bronchopneumonia caused by reovirus infection was clearly exacerbated by DON. Viral-induced elevations of protein, monocyte chemoattractant protein-1, TNF-α, and inflammatory cells in the bronchioalveolar

lavage fluids (BALF) and upregulation of reovirus-specific immunoglobulin A (IgA) in BALF, fecal pellets, and serum also were markedly enhanced by the presence of DON. The overall observed effects suggested that DON compromised resistance to respiratory viral infection (Li et al., 2007).

DON (5, 10, and 25 mg/kg [0.02, 0.04, and 0.84 mmol/kg]), administered to male rats by intraperitoneal (i.p.) injection, affected platelets and white and red blood cells. It also induced necrosis of hepatocytes, lymphocytes, and pancreatic aciner cells assessed 3 and 24 hours post-injection (Robl et al., 2005 abstr.).

### 9.1.4 Short-Term and Subchronic Exposure

Details of the following studies are presented in **Table 1**.

Overall, studies showed that short-term and subchronic exposure to DON decreased body weight, weight gain, and feed consumption in rats and mice. Hematological effects also were observed.

Conflicting results are observed for the effect of DON on organ weights. Gouze et al. (2003 abstr.) and Collins et al. (2004 abstr., 2006 [PMID:16325976]) reported that spleen and liver weights and the liver-body and kidney-body weight ratios increased in Sprague-Dawley rats gavaged with DON. In other studies, Gouze et al. (2006 [PMID:16209902]) and Sprando et al. (2005 [PMID:15721211]) reported no effect on organ weight or organ-body weight ratios in rats and mice. Kim et al. (2007) reported dose-dependent decreases in the relative weights of thymus, seminal vesicle/prostate, and testes while the relative weights of liver and left adrenal gland increased dose-dependently. DON induced lesions in the non-glandular stomach, and caused thymic lymphoid depletion, increased incidences and mean severity of splenic hematopoiesis, and increased mean severity of sternal bone marrow adipocyte deposition in rats at the highest dose (Sprando et al. 2005 [PMID:15721211]).

The impact of stress on the effects of DON were evaluated in male BALB/c mice. Mice (12 animals/group) received 0 or 2 mg/kg bw in the diet for 14 days. Mice ran a treadmill until they could no longer maintain the pace with prodding to induce stress. No significant difference between final body weight, average daily feed intake, weight gain, or total number of spleen cells was noted between the treatment and control group. DON with exercise inhibited plaque formation in spleen cells of exercised mice and significantly inhibited Con-A stimulated lymphocyte proliferation in non-exercised mice. DON also was shown to modulate interleukin expression (Landgren et al., 2006).

### 9.1.5 Chronic Exposure

Female B6C7$F_1$ mice (7 weeks old) were fed experimental diets for 16 weeks that contained DON (20 mg/kg [67 µmol/kg]). DON reduced the mean daily food consumption (2.94 ± 0.66 g vs. 3.6 ± 0.48 g), the mean body weight gain (2.76 ± 0.84 g vs.12.94 ± 1.68 g), and total body weight, and increased serum immunoglobulin A (IgA) levels in treated vs. control mice starting 8 weeks after diet initiation. Serum IgA immune complex (IgA-IC) levels and mesangial IgA deposition increased starting 16 weeks after diet initiation. DON also increased *ex vivo* IgA secretion from the spleen and Peyer's patches (Shi and Pestka, 2006 [PMID:16524712]).

## Table 1. Short-Term and Subchronic Exposure to DON

| Species, Strain, and Age, Number, and Sex of Animals | Route, Dose, Duration, and Observation Period | Results/Comments | Reference |
|---|---|---|---|
| Mice, C57BL/6-J (WT), TNFR1-KO, and TNFR2-KO, 8-wk- old, 6M/strain | oral (diet); 10 ppm [34 µmol/kg] for 12 wk | Feed intake and body weight gain were significantly decreased compared to controls. By the end of the treatment period, overall weight gains were suppressed by 53, 64, and 67% in TNFR1, TNFR2, and WT groups, respectively. Feed conversion efficiency was decreased fourfold in WT mice and twofold in TNFR-KO mice at weeks 6 and 12. | Pestka and Zhou (2002 [PMID:12176089]) |
| Mice, C57BL/6-J, age, number, and sex n.p. | oral; 0.014-8.870 mg/kg bw [0.047-29.93 µmol/kg bw; equiv. to 0.05-25 ppm] in a gum suspension for 3 days/wk for 4 wk | Body weight gain was not affected, but DON significantly reduced spleen weight and increased liver weight at the high dose. DON also increased sodium, phosphate, creatinin, calcium, and total protein levels. | Gouze et al. (2003 abstr.) |
| Mice, C57BL6, 6-wk-old, 10 M/group | oral; 0.071 and 0.355 mg/kg [0.24 and 1.20 µmol/kg] bw for 3 days/wk for 4 wk | No significant effect on body weight gain, liver and spleen weights, liver protein content, plasma biochemistry, or cytokine release (*in vitro*). Caused significant increase in IgA concentration (33-41%) and induction of Phase I and Phase II liver metabolic enzymes. | Gouze et al. (2005 [PMID:16375817]) |
| Mice, C57BL6, 6-wk-old, 10M/group | oral; 0.014-1.774 mg/ kg bw [0.047-5.987 µmol/kg; equivalent 0.05-5 ppm daily average intake] for 3 days/wk for 4 wk | No significant effect on body weights, liver weights, and hepatic microsomal and cytosolic proteins. | Gouze et al. (2006 [PMID:16209902]) |

**Table 1. Short-Term and Subchronic Exposure to DON (Continued)**

| Species, Strain, and Age, Number, and Sex of Animals | Route, Dose, Duration, and Observation Period | Results/Comments | Reference |
|---|---|---|---|
| Mice, B6C3F$_1$, age and number n.p., M | oral; 0.83-7.5 mg/kg [2.8-25 µmol/kg] bw for 8 days | Body weight gain was significantly reduced at the highest dose. Anorexia, ataxia, fur crudeness, and lack of vigor also were observed at the highest dose DON group. Relative organ weights of thymus, seminal vesicle/prostate, and testes were dose-dependently reduced while the relative weights of liver and left adrenal gland were dose-dependently increased. Atrophy of thymus, seminal vesicle/prostate, and testes was observed. Additonally, submucosal edema and ulceration in stomach and lymphocytes depletion in thymus cortex were seen.<br><br>The number of white blood cells and platelets, and hemoglobin content was reduced. Prothrombin time and activated partial thromboplastin time were prolonged in a dose-dependent manner. Additionally, total serum protein, globulin, blood urea nitrogen, cholesterol, and testosterone were reduced. Comparatively, fibrinogen content was elevated at the high dose and total bilirubin and albumin/globulin ratio increased. Alkaline phosphatase activity was decreased while alanine aminotransferase activity was increased. | Kim et al. (2007) |
| Rats, Wistar, age and number, n.p., M | s.c., 1 mg/kg bw [3 µmol/kg bw] for 3 days, sacrificed 24 h after last injection | Rats exhibited increases in blood insulin, glucose, and free fatty acids. A statistically significant increase in glycogen (3.52 mg/g wet tissue vs. 2.81 mg/ g wet tissue [controls]) and reduction in triglycerides [11.91 µmol/g wet tissue vs. 19.13 µmol/g wet tissue [controls]) were observed in muscle tissue (Szkudelska et al., 2002). Similar effects were observed via i.p. infusion. | Prelusky (1997; cited by Szkudelska et al., 2002 [PMID:12368054]) |

## Table 1. Short-Term and Subchronic Exposure to DON (Continued)

| Species, Strain, and Age, Number, and Sex of Animals | Route, Dose, Duration, and Observation Period | Results/Comments | Reference |
|---|---|---|---|
| Rats, Sprague-Dawley, age n.p., 24F/group | oral; 0-5.0 mg/kg bw [0-0.02 mmol/kg bw] on GD 6-19 | All females survived to GD 20. DON caused a dose-related increase in the number of females with excessive salivation. At the higest dose, diarrhea (2 rats), vaginal discharge (2 rats), emaciation (1 rat), and rough coat (1 rat) were observed. Feed consumption was significantly decreased in the 2.5 and 5 mg/kg bw group during gestation days 8-14 and 6-19, respectively. Fluid consumption was significantly increased at 2.5 mg/kg bw on gestation days 13–19. Overall mean body weight gain on days 0–20 decreased in a dose-related manner.<br><br>Dose-related increases in maternal liver-to-body weight were observed at doses >0.5 mg/kg bw. Kidney-to-body weight ratio increased at 1.0 and 5.0 mg/kg bw. The NOAEL for maternal toxicity was 0.5 mg/kg bw based on the dose-related increase in liver-to-body weight ratio at 1 mg/kg bw. | Collins et al. (2004 abstr., 2006 [PMID:16325976]) |
| Rats, Sprague-Dawley, age n.p., 15-16 M/ group | oral; 0.5-5 mg/kg [2 µmol/kg] bw per day for 28 days | Significant decrease in body weight gain observed at 2.5 mg/kg bw during dosing days 15–29 and for the entire dosing period (Days 0–29). Body weight gain also was decreased during the entire dosing period in rats given 5.0 mg /kg bw. Average feed consumption decreased during dosing days 22–29 and throughout the treatment period, respectively. Body weight only was significantly decreased in the 5.0 mg/kg bw dose groups. Decreased heart-to-brain and kidney-to-brain weights were observed in the 5.0 mg/kg bw and 2.5 and 5.0 mg/kg bw groups, respectively. No significant effect on organ-body weight ratios was observed. Lesions seen in the non-glandular stomach of animals in the highest dose group showed the presence of. Additional findings included the presence of thymic lymphoid depletion, increased incidences and mean severity of splenic hematopoiesis, increased mean severity of sternal bone marrow adipocyte deposition. | Sprando et al. (2005 [PMID:15721211]) |

Abbreviations: bw = body weight; F = female(s); GD = gestational day(s); h = hour(s); i.p. = intraperitoneal; M = male(s); NOAEL = no-observed-adverse-effect level; n.p. = not provided; s.c. = subcutaneous(ly); wk = week(s)

### 9.1.6 Synergistic/Antagonistic Effects

In *Salmonella typhimurium* strain TA98, the combination of DON and aflatoxin B1 (AFB1) had a greater mutagenic effect than AFB1 alone (Smerak et al., 2001). In primary rat hepatocytes, the combination induced DNA synthesis at the S phase of the cell cycle (Li and Guo, 2000 [PMID:12520966]). In a yeast (*Kluyveromyces marxianus*) bioassay, DON plus nivalenol (NIV) exhibited a synergistic response; antagonism was observed with DON plus T-2 toxin (Madhyastha et al., 1994 [PMID:7801350]). In mouse fibroblast L929 cells, a mixture containing DON, NIV, T-2 toxin, zearalenone, and fumonisin B1 produced greater inhibition of DNA synthesis than with treatment of each mycotoxin alone. Additionally, a synergistic interaction between DON and NIV was seen (Tajima et al., 2002 [PMID:11955675]). A synergistic carcinogenic effect was observed in NIH mice when Sterigmatocystin and DON were both administered. The number of animals with lung adenocarcinomas and glandular stomach dysplasia increased (Huang et al., 2004 [PMID:15733384]). DON toxicity in human chondrocytes was reversed by selenium (Yue et al., 2005).

In $B6C3F_1$ mice, coadministration of lipopolysaccharide (LPS) and DON significantly upregulated splenic interleukin (IL)-1β mRNA and plasma IL-1β protein expression compared to treatment with the toxins alone (Islam and Pestka, 2003). Mice and murine RAW 264.7 macrophages were sensitized to DON-induced proinflammatory gene expression (e.g., IL-6, IL-1β) by pre-exposure to LPS and Toll-like receptor agonists, respectively (Islam and Pestka, 2006 [PMID:16009389]; Pestka and Zhou, 2006 [PMID:16687389]). Synergistic interactions were observed between DON and pro-inflammatory agents (e.g., IL-1β) on NF-κB activation and IL-8 secretion (Maresca et al., 2008 [PMID:18308354]; Van De Walle et al., 2008 [PMID:18343055]). Fish oil suppressed DON-induced mitogen-activated protein kinase (MAPK) phosphorylation and IL-6 expression in mice but attenuated DON-induced IgA nephropathy (Moon and Pestka, 2003b [PMID:14690764]; Pestka et al., 2002; Shi and Pestka, 2006 [PMID:16524712]). Decosahexaenoic acid and eicosapentaenoic acid also suppressed the effect, and decosahexaenoic acid suppressed DON-induced IL-6 transcription (Jia et al., 2004, 2006 [PMID:16424113]). DON also increased severity of reovirus infection in mice (Li et al., 2005 [PMID:15958657]).

### 9.1.7 Cytotoxicity

In mouse thymocytes *in vivo*, DON (0.5-8.0 mg/kg [2-27 µmol/kg]) dose-dependently induced significant increases in apoptosis rates compared to controls. Characteristic DNA ladder, chromatin condensation, and nuclear budding phenomenon were observed in the cells. In addition, DON (4 and 8 mg/kg [13 and 27 µmol/kg]) significantly decreased the proliferation indexes of the treated thymocytes *in vim* (Li et al., 2002).

In Swiss mouse 3T3 fibroblasts, 24-hour incubation with DON (78-2500 ng/mL [0.2-8.4 µM]) resulted in an $IC_{50}$ value (concentration inhibiting the incorporation of BrdU in DNA by 50%) of ~444 ng/mL. The $IC_{50}$ value for the de-epoxide of DON was 52 times higher based on mass concentration and 55 times higher based on molar concentration (Sundstol Eriksen et al., 2004 [PMID:15019186]).

Numerous studies have been conducted in a variety of cell lines to assess the cytotoxic effects of DON. Cytotoxic effects have been observed in pre-B lymphocyte REH, human, pre-T

lymphocyte Jurkat, hamster kidney-derived BHK21, mouse hepatoma MH-22a, HEp-2, CHO-K1, renal proximal tubule epithelial, normal human lung fibroblast, human dendritic, Caco-2, lung A549, monocytic U937, Hep-G2, MRC-5, human erythroleukemia K562, human gastric carcinoma cell line HGC-27, human peripheral blood mononuclear cells, and human primary hepatocytes (Baltriukiene et al., 2007 [PMID:17411352] Calvert et al., 2005 [PMID:15883728]; Cetin and Bullerman, 2005 [PMID:15778016]; Humpf and Konigs, 2008 abstr.; Hymery et al., 2006 [PMID:16517116]; Instanes and Hetland, 2004 [PMID:15369845]; Ivanova et al., 2006 [PMID:16730043]; Konigs et al., 2007 [PMID:17825972], 2008 [PMID:18618482]; Liu et al., 2007; Minervini et al., 2004 [PMID:14630058]; Zhou et al., 2006a [PMID:17166419], 2006b [PMID:17166420]).

Sahu et al. (2008 [PMID:18300328]) showed that rat liver clone-9 cells treated with DON (up to 100 µg/mL) exhibited a dose-dependent increase in cell death (decreased rate of reazurine reduction and a decreased cellular double-stranded DNA content). In male Sprague-Dawley rats (9 weeks old) treated with a single i.p. injection of DON (10 mg/kg bw), hepatocyte necrosis was observed in 3 of 4 and 1 of 4 rats at 3 and 24 hours post-injection, respectively. Necrosis was not seen in any of the four mice maintained for 72 hours after treatment.

## 9.2 Reproductive and Teratological Effects

In three-month-old nulliparous female NMRI mice, i.p. injection of DON (3.3, 4.2, 5, or 10 mg/kg [11, 14, 17, or 34 µmol/kg] on gestation days 7 and 9 or 1.6, 2.5, or 3 mg/kg [5.4, 8.4. or 10 µmol/kg] daily on gestation days 7-10) produced high maternal deaths at the two higher doses. In embryos, the number of resorptions was dose-dependently increased in treated animals compared to controls. Skeletal abnormalities (mostly in the axial skeleton) were observed. Exencephaly was mainly seen at 75 or 100 µg/30 g during the four-day treatment. At the higher dose and shorter exposure period, neural arch defects or fusion were mostly seen. In both experiments, vertebral bodies showed various deformities (destruction or division), hemivertebrae (except with 75 µg/30 g given for four days), and fused, branched, and/or cervical ribs. In the two-day experiment, the effects were dose-dependent, and in the four-day experiment, the incidences were lower (Debouck et al., 2001 [PMID:11482540]).

Sprague-Dawley female rats administered DON (0.5, 1, 2.5, or 5 mg/kg [2, 3, 8, or 17 µmol/kg]) via oral gavage on gestation days 6-19 showed decreased mean gravid uterine weight at the highest dose tested (5 mg/kg bw/day). At 2.5 and 5 mg/kg bw/day, average fetal body weight, crown-rump length, and ossification of fetal vertebrae were significantly decreased. At 5 mg/kg bw/day, 52% of litters (12/23) were resorbed. Additionally, significant increases were observed in the average number of early and late deaths per litter, the incidence of runts, and the incidence of misaligned and fused sternebrae, while the ossification of fetal sternebrae, centra, dorsal arches, vertebrae, metatarsals, and metacarpals was significantly decreased. [Note: The authors noted that these effects may be secondary to maternal toxicity and the reduced size of the fetuses.] The no-observed-adverse-effect level (NOAEL) for fetal toxicity was determined to be 1 mg/kg based on the general reduction in fetal development at higher doses; that for maternal toxicity was established at 0.5 mg/kg based on the increased liver-body weight ratio at 1 mg/kg. DON was considered a teratogen at 5 mg/kg bw/day based on the anomalous development of the sternebrae (Collins et al., 2004 abstr., 2006 [PMID:16325976]).

Male Sprague-Dawley rats were treated with DON (0.5, 1, 2.5, or 5 mg/kg [2, 3, 8, or 17 µmol/kg] bw) daily via gastric intubation for 28 days. Epididymal (right and left) and seminal vesicle weights (expressed per gram of body weight and brain weight) were significantly reduced in animals treated with 2.5 and 5.0 mg/kg bw. Decreased prostate weight (expressed per gram of body weight and brain weight), spermatid numbers, cauda epididymal sperm numbers, and cauda epididymal sperm numbers/gram cauda epididymis were observed in the 5.0 mg/kg bw dose group. Incresaed sperm tail abnormalities (broken tails) also were observed in the 5.0 mg/kg bw dose group while sperm swimming speed was increased only in the 2.5 mg/kg bw dose group. Serum concentrations of follicle-stimulating hormone, luteinizing hormone, and testosterone were modulated in a dose-dependent manner. Increases in germ cell degeneration, sperm retention and abnormal nuclearmorphology were observed at doses >2.5 mg/kg bw (Sprando et al., 2005 [PMID:15721211]).

A number of current studies focus on pigs. In swine herds exposed to DON (3.14 ppm [10.6 µg/mg]) in feed, reproductive problems such as swollen vulvas, vaginal and rectal prolapses, infertility or decreased fertility, prolonged estrous cycle or failure to return to estrus, abortion, and small litter size were reported in 50% field cases (Cote et al., 1984 [PMID:6230342]). When sows were orally given DON (~2600 µg/kg [8.8 µmol/kg] in test rations), lesions, necroses, and edemas were seen in the skin, ears, and tails of piglets in the second and third reproduction cycles, as well as incoordination and deformation of extremities (Jadamus and Schneider, 2002). Female pigs orally administered DON (3.0 mg/kg [10 µmol/kg] feed) on days 8-255 of the rearing period had delayed onset of puberty and a predisposition to endometritis (Horugel and Vergara, 2003). In another study, prepubertal piglets were given diets containing DON (0.2, 0.8, 1.0, 1.9, or 3.9 mg/kg [0.7, 2.7, 3.4, 6.4, or 13 µmol/kg]). At the high dose, significant increases in the mean weight of the uteri were observed at the time of slaughtering (Doll et al., 2003a [PMID:15595624]). In porcine cumulus oocyte complexes, DON (0.94, 1.88, 3.75, or 7.5 µM [0.28, 0.557, 1.11, or 2.2 µg/mL]) dose-dependently decreased maturation (telophase 1 and metaphase 2) rates and increased degeneration rates after 48 hours culture *in vitro* (Alm et al., 2002 [PMID:12423645]).

### 9.3 Carcinogenicity

The International Agency for Research on Cancer (IARC) concluded that "[t]here is inadequate evidence in experimental animals for the carcinogenicity of deoxynivalenol." Overall, it was placed in Group 3, "not classifiable as to its carcinogenicity to humans" (IARC, 1993).

In a previous two-year carcinogenicity study in mice, dietary administration of DON did not result in an increased incidence of neoplasms in males or females; in males, there was a decreased incidence of liver neoplasms, probably a result of lower body weights (Iverson et al., 1995 [PMID:8732880]). Details of this study are also provided in the JECFA monograph (JECFA, 2001d).

Gastric intubation of NIH mice with DON (1.5 µg/kg [5.1 nmol/kg] bw) three times a week for 24 weeks induced lung adenocarcinoma (37.5% of mice) and dysplasia of glandular stomach (25.0% of mice) (Huang et al., 2004 [PMID:15733384]).

### 9.4 Initiation/Promotion Studies

DON did not initiate or promote cell transformation in a short-term test using v-Ha-*ras*-transfected BALB/3T3 cells. These results agree with previous initiation and promotion studies in experimental animals (Sakai et al., 2007 [PMID:17499015]).

### 9.5 Anticarcinogenicity

No additional data were available.

### 9.6 Genotoxicity

In Chinese hamster V79 cells, DON fractions from samples of wheat (30 ng/mL [0.10 µM]), barley (200 ng/mL [0.675 µM]), and corn (300 ng/mL [1.01 µM]) induced chromosome aberrations, mostly chromatid breaks (Hsia et al., 2004 [PMID:15254715]).

DON (1-10 µmol) induced DNA damage in Vero cells (increased number of cells with long tails, tail DNA, tail length, and tail motion) in a dose- and time-dependent manner. Short-term incubations (4 hours) mainly induced an increase of the number of DNA fragments while longer inubation time (16 hours) mainly caused small size DNA fragments (Lin and Sun, 2004; cited by Ma and Guo, 2008).

Results from the Comet assay showed that DON increased mean tail moment in human Caco-2 cells in a dose-dependent manner at concentrations (0.01-0.05 µM [3-15 ng/mL]) that did not induce apoptosis. Furthermore, dividing cells exhibited greater sensitivity to DON than differentiated cells (Bony et al., 2006 [PMID:16828243]).

### 9.7 Cogenotoxicity

No additional data were available.

### 9.8 Antigenotoxicity

No additional data were available.

### 9.9 Immunotoxicity

Numerous immunotoxicity studies are cited in the JECFA monograph under the following topics: altered host resistance and humoral and cell-mediated responses, altered serum IgA levels, IgA-associated nephropathy, cytokine expression, and apoptosis in lymphoid tissue (JECFA, 2001a). Copious studies have since been conducted (e.g., Chung et al., 2003a [PMID:14644621], 2003b [PMID:12604170]; Gray and Pestka, 2007 [PMID:17636245]; Kinser et al., 2005 [PMID:15681167]; Pestka and Zhou, 2000 [PMID:10942317]; Sugita-Konishi and Pestka, 2001 [PMID:11766169]; Uzarski et al., 2003 [PMID:14597125]; Van De Walle et al., 2008 [PMID:18343055]; Wang et al., 2000 [PMID:12520964]; Wong et al., 2002 [PMID:12167214]; Yang et al., 2000a [PMID:10652249], 2000b [PMID:10764628]; Zhou et al., 1999 [PMID:10344227]). Some of the more recent studies are presented in **Table 2**.

## Table 2. Immunotoxicity Studies for DON

| Species, Strain, and Age, Number, and Sex of Animals | Chemical Form and Purity | Route, Dose, Duration, and Observation Period | Results/Comments | Reference |
|---|---|---|---|---|
| ***In Vivo* Assays** | | | | |
| Mouse, B6C3F$_1$, 8- to 10-wk-old, 2M/dose group | DON, purity n.p. | oral (gavage); single dose of 1, 5, 25, or 100 mg/kg [3, 17, 84, or 337 µmol/kg]; killed after 15, 30, 60, and 120 min | MAPK studies: MAPK phosphorylation was induced. At 1 mg/kg, JNK 1/2, ERK 1/2 and p38 phosphorylation was induced. At 5 mg/kg, maximal phosphorylation of all three occurred. | Zhou et al. (2003) |
| Mouse, B6C3F$_1$, 8- to 10-wk old, 2M/dose group | DON, purity n.p. | oral (gavage); 25 mg/kg [84 µmol/kg]; killed at 0.5, 1.5, 4, and 8 h (kinetic studies) or at 0.5, 1.5, and 4 h | Transcription-factor studies: JNK 1/2 and p38 phosphorylation was largely reduced at 60 and 120 min. ERK 1/2 activation was more prolonged, with maximal phosphorylation detection in spleen and thymus from 15-60 min. At 1 mg/kg, MAPK phosphorylation was rapidly induced in spleen.<br>DON activated the following activities and nuclear translocation: AP-1 binding, which involved only Jun protein dimers; C/EBP binding at 0.5 h (mainly through increased nuclear C/EBPβ), followed by a nearly complete loss of detectable binding activity; CREB binding (mainly nuclear CREB-a and ATF-2); and NF-κB binding (late transient increase in the activity, correlating with increased nuclear p50 and cRel). | Zhou et al. (2003) |
| Mouse, B6C3F$_1$, 8- to 10-wk old, 2M/dose group | DON, purity n.p. | oral (gavage); single dose of 12.5 mg/kg [42 µmol/kg]; measured at 3, 6, and 9 h | Cytokine mRNA studies: Proinflammatory cytokine mRNA expression was transiently induced. TNF-α mRNA expression was 3x and 2x control values at 3 and 6 h, respectively. Splenic IL-1β mRNA expression was 1.8x and 1.4x controls at 3 and 6 h, respectively. IL-6 mRNA was 17x and 2x controls at 3 and 6 h, respectively. At 9 h, all levels returned to control levels. | Zhou et al. (2003) |
| Mouse, B6C3F$_1$, 8- to 9-wk-old, 12F/dose group | DON, purity n.p. | oral (diet); 25 ppm [84 µmol/kg] for 16-24 wk or for 8 wk (withdrawal groups) | Serum IgE in animals in both groups was increased 2- to 5-fold compared to controls at 12-24 wk. In mice in the withdrawal group, serum IgE levels were 2.4-, 4-, 4.9-, and 2-fold that of controls at 12, 16, 20, and 24 wk, respectively. Maximum IgE levels reached 1000 to 1600 ng/mL.<br>Serum IgA was increased at 8 wk during DON administration and remained significantly elevated over control values after DON withdrawal. At wk 16, mice in the withdrawal group had significantly more serum IgG than the control group. | Pestka and Dong (1994 [PMID:8005381]) |

## Table 2. Immunotoxicity Studies for DON (Continued)

| Species, Strain, and Age, Number, and Sex of Animals | Chemical Form and Purity | Route, Dose, Duration, and Observation Period | Results/Comments | Reference |
|---|---|---|---|---|
| Mouse, B6C3F$_1$, 7-wk-old, 3M/dose group | DON, purity n.p. | oral (gavage); single dose of 1, 5, or 25 mg/kg [3, 17, or 84 µmol/kg] bw | At 5 and 25 mg/kg, COX-2 mRNA levels were significantly increased in spleens and Peyer's patches; maximum expression was seen at 2 h for both organs. At 25 mg/kg, IL-6 mRNA was induced in both but peak expression was at 2-4 h. | Moon and Pestka (2003a [PMID:12649040]) |
| Mouse, B6C3F$_1$, 8- to 10-wk-old, 13-14F/dose group | DON (from *F. graminearum* culture and "purified") | oral; 25 ppm [40 µmol/kg] in diet for 12 wk<br>There were four treatment groups: mice fed standard diet, mice fed standard diet containing DON, CT-challenged mice fed standard diet, and CT-challenged mice fed standard diet containing DON. | After 12 wk, DON-treated mice had a ~7-fold increase in total serum IgA, and DON-treated CT mice had an 8-fold increase. After 16 and 20 wk, casein-specific IgA was 10-fold greater in DON-treated mice compared to controls. At 22 wk, casein-specific IgA was similarly elevated in CT-immunized mice fed DON. CT-specific serum IgA levels did not differ between CT-immunized mice fed DON and those fed a standard diet. CT-specific IgA levels were 5-fold greater in unimmunized mice fed DON versus those fed standard diet.<br>After 8 wk, CT-specific IgG was ~70-fold less in CT-immunized mice fed DON versus CT-immunized controls, and casein-specific IgG was decreased 15-fold and 5-fold in unimmunized and CT-immunized mice fed DON, respectively, compared to their corresponding controls. | Pestka et al. (1990 [PMID:2296780]) |
| Mouse, C57/BL6, age, number, and sex n.p. | DON, "pure" | oral; 0.014, 0.071, 0.355, 1.774, or 8.870 mg/kg [0.047, 0.24, 1.20, 5.987, or 29.93 µmol/kg] bw (equivalent to 0.05, 0.2, 1, 5, or 25 ppm) for 3 days/wk x 4 wk | Serum IgA levels were significantly increased at 0.071 and 0.355 mg/kg, and serum IgM levels were decreased at 8.87 mg/kg. | Gouze et al. (2003 abstr.) |
| Mouse, C57BL/6-J (WT controls), TNFR1-KO, and TNFR2-KO, 8-wk-old, 6M/dose group | DON (produced in *F. graminearum* R6576 cultures and "purified") | oral; 10 ppm [34 µmol/kg] in AIN-76A diet administered for 12 wk; killed at the end of treatment period | WT and TNFR2-KO mice: serum IgA concentrations significantly increased compared to controls at wk 8 and 12. By wk 12, there was a significant difference between TNFR1-KO mice fed DON and those fed control diet. IgA in TNFR1-KO vs. WT mouse was significantly less at all time points and significantly less than for TNFR2-KO mice. Serum IgA-IC was significantly increased at 12 wk in all treated mice vs. controls. Levels were 5- and 3-fold higher for WT and TNFR2-KO than for TNFR1-KO mice, respectively.<br>Significantly more mesangial IgA was found in kidneys of WT and TNFR2-KO mice after 12 wk. only slight elevation reported for TNFR1-KO mice. | Pestka and Zhou (2002 [PMID:12176089]) |

## Table 2. Immunotoxicity Studies for DON (Continued)

| Species, Strain, and Age, Number, and Sex of Animals | Chemical Form and Purity | Route, Dose, Duration, and Observation Period | Results/Comments | Reference |
|---|---|---|---|---|
| Mouse, strain, age, number, and sex n.p. | DON, purity n.p. | oral, 25 mg/kg [84 µmol/kg] in the presence or absence of feed containing (n-3) PUFA | DON induced expression of IL-1α, IL-1β, IL-6, IL-11, MCP-1, MCP-3, CINC-1, MIP-2, c-Fos, Fra-2, c-Jun, JunB, MKP1, and CnAβ. Expression of most proteins peaked at 2-4 hours. PUFA suppressed DON-induced expression of IL-1α, IL-6, IL-11, MCP-1, MCP-3, MIP-2 and Fra-2 at 8 h and increased expression of MKP1 and CnAβ. | Kinser et al. (2005 [PMID:15681167]) |
| ***In Vitro* Assays** | | | | |
| RAW 264.7 murine macrophage cells | DON, purity n.p. | exposure to 10, 50, 100, or 250 ng/mL [0.034, 0.17, 0.337, or 0.844 µM] for 24 h | DON induced COX-2 gene expression. At 50-250 ng/mL, the production of PGE2, a major COX-2 metabolite, was significantly enhanced. At 100 and 250 ng/mL, dose-dependent increases in COX-2 protein were seen at 15 h, while increases in COX-2 mRNA were seen at 2 h. DON also increased COX-2 mRNA stability. At 250 ng/mL, MAPK (ERK, p38, and JNK) phosphorylation was enhanced. | Moon and Pestka (2002) |
| RAW 264.7 murine macrophage cells | DON, purity n.p. | incubated with 0-250 ng/mL [0-0.844 µM] in the presence and absence of selective inhibitors | At 100-250 ng/mL, DON induced COX-2 protein expression. LPS-induced IL-6 production was superinduced by cotreatment with 100 ng/mL DON. | Moon and Pestka (2003a [PMID:12649040]) |
| WEHI-231 immature B cells | DON, purity, n.p. | exposure of cells to 10-1000 ng/mL [0.337-3.375 µM] for 18 h | DON induced cytotoxicity and apoptosis at 100-1000 ng/mL.<br>$EC_{50}$ = 500 ng/mL for apoptosis and 300 ng/mL for cytotoxicity | Uzarski and Pestka (2003 [PMID:14710595]) |
| Caco-2 cells | DON, purity n.p. | Incubation with 250-10,000 ng/mL [0.844 – 33.7 µM] in the presence or absence of pro-inflammatory stimuli for 24 h | Dose-dependent increases in NF-κB activity and IL-8 secretion observed at highest concentration tested. Inhibitor κB phosphorylation occurred at <500 ng/mL. Synergistic interactions observed between DON and stimuli. | Van De Walle et al. (2008 [PMID:18343055]) |
| U937 human monocyte cells | DON, purity n.p. | n.p. | IL-8 expression, as measured by luciferase expression, was upregulated in U937 cells exposed to 1 µg/mL DON. This effect was blocked by caffeic acid phenethyl ester. Mutation of the NF-κB and activator protein-1 binding site significantly inhibited and increased DON-induced luciferase expression, respectively. DON also increased p65 binding by 21-fold and decreased p52 binding. | Gray and Pestka (2007 [PMID:17636245]) |

## Table 2. Immunotoxicity Studies for DON (Continued)

| Species, Strain, and Age, Number, and Sex of Animals | Chemical Form and Purity | Route, Dose, Duration, and Observation Period | Results/Comments | Reference |
|---|---|---|---|---|
| human lymphocytes | DON, purity n.p. | incubation with 100-400 ng/mL [0.337-1.35 µM] for 72 h; cytokine production was followed for 8-9 days at 200 and 400 ng/mL [0.675 and 1.35 µM] | *Cell proliferation*: At 216 ng/mL, cell proliferation was inhibited 50%. At 200 and 400 ng/mL in PHA-stimulated lymphocytes, IL-2 levels were up to 12-fold higher compared to controls, IL-4 levels were only slightly increased, and IL-6 levels were slightly inhibited.<br>*Cytokine production*: At 200 ng/mL, there was a simultaneous increased in IL-2 levels (17- to 25-fold) and IFN-γ. IL-6 levels were slightly decreased. At 400 ng/mL, IL-2 levels were significantly increased up to six days post-treatment; the effects on IL-4 and IL-6 were less marked. | Meky et al. (2001 [PMID:11434990]) |
| peripheral mononuclear blood leukocytes | DON, purity n.p. | incubation with 10, 50, 100, 250, or 500 ng/mL [0.034, 0.17, 0.337, 0.844, or 1.69 µM] for 6 h | The following parameters were significantly induced by DON:<br>**IL-6 ~9.5-fold @ 250 ng/mL**<br>**~14-fold @ 500 ng/mL**<br>**IL-8 ~8.5-fold @ 250 ng/mL**<br>**~3-fold @ 500 ng/mL**<br>**TNF-α ~1-fold @ 10 ng/mL**<br>**~1-fold @ 250 ng/mL**<br>**~4-fold @ 500 ng/mL** | Penner et al. (2003 abstr.) |
| human peripheral blood mononuclear cells | DON, purity n.p. | incubation with 100, 1000, or 5000 ng/mL [0.337, 3.375, or 16.87 µM] for 4 h | DON significantly and dose-dependently inhibited T and B cell proliferation. Significant inhibitory effects on antibody-dependent cellular cytotoxicity reaction were seen. DON exhibited dose-dependent inhibitory effects on NK cell activity. | Berek et al. (2001 [PMID:11259866]) |

Abbreviations: AP = activating protein; ATF = activator transcription factor; C/EBP = CCAAT enhancer-binding protein; COX-2 = cyclooxygenase-2; CREB = cyclic AMP response element-binding protein; CT = cholera toxin; $EC_{50}$ = effective concentration producing 50% process; ERK 1/2 = extracellular signal regulated protein kinase 1 and 2; h = hour(s); IFN = interferon; Ig = immunoglobulin; JNK 1/2 = p54 and -46 c-Jun *N*-terminal kinase 1 and 2; LPS = lipopolysaccharide; M = male(s); MAPK = mitogen-activated protein kinase; MCP = monocyte chemoattractant proteinmin = minute(s); MIP = macrophage inflammatory protein; NF-κB = nuclear factor-κB; NK = natural killer; n.p. = not provided; PGE2 = prostaglandin E2; PHA = phytohemagglutinin; PUFA = polyunsaturated fatty acids; TNF = tumor necrosis factor; wk = week(s)

*In vivo* in mice studies show that DON induced MAPK phosphorylation and cytokine mRNA expression, as well as serum IgA and IgE levels (Pestka, 2003 [PMID:12676476]; Pestka and Dong, 1994 [PMID:8005381]; Zhou et al., 2003). *In vivo* and *in vitro* studies show that it also superinduced proinflammatory cytokine gene expression; its effects on tumor necrosis factor-α (TNF-α) and IL-6 gene expressions in LPS-stimulated macrophages have been conducted regarding the underlying molecular mechanisms involved (Wong et al., 2001 [PMID:11295263]). In TNFR1-KO mice, serum IgA and IgA-IC, and kidney IgA deposition were markedly decreased compared to WT and TNFR2-KO groups when given diets containing DON (Pestka and Zhou, 2002 [PMID:12176089]). Immunization of mice with cholera toxin had no effect on total serum IgA (Pestka et al., 1990 [PMID:2296780]). DON induced cyclooxygenase-2 (COX-2) gene expression *in vivo* and *in vitro*, which played a part in the induction of IL-6 gene expression (Moon and Pestka, 2002, 2003a [PMID:12649040]).

Additional *in vitro* studies showed that DON inhibited nuclear protein binding to NRE-A, an IL-2 promoter negative regulatory element, in murine lymphoma EL-4 T cells, induced cytotoxicity and apoptosis in WEHI-231 B cells, induced p38 activation, and increased IL-8 production (Bae and Pestka, 2008 [PMID:18502741]; Moon et al., 2007 [PMID:17707346]); Uzarski and Pestka, 2003 [PMID:14710595]; Yang and Pestka, 2002 [PMID:11893416]). Zhou et al. (2005 [PMID:15976193]) showed that the competing immunosuppressive and immunostumulatory effects of DON were mediated through competing apoptotic and survival pathways.

### 9.10 Other Data

In adipocytes isolated from male Wistar rats, DON (20 µM [5.9 µg/mL]) slightly stimulated basal lipogenesis but had no effects on insulin-induced lipid synthesis and lipolysis. DON also did not affect cell viability (Szkudelska et al., 2002 [PMID:12368054]).

In RAW 264.7 murine macrophages, PP1 (Src-family-tyrosine kinase inhibitor selective for Hck) and 2-AP (2-aminopurine) additively inhibited DON-induced caspase-3 activity and apoptosis, p53-binding activity, and MAPK (p38, ERK, and JNK) phosphorylation. PFTα (p53 inhibitor) canceled DON-induced caspase-3 activity and apoptosis, while SB 203580 (p38 inhibitor) canceled DON-induced p21 phosphorylation and p53 binding activity. Additionally, p38 inhibition blocked DON-induced apoptosis, ERK inhibition promoted DON-induced apoptosis, and JNK inhibition caused no effect (Zhou and Pestka, 2003 abstr.). In another study using macrophages, PD98059 (MEK1/2 inhibitor that inhibits ERK activation) and SB 203580 significantly reduced DON-induced prostaglandin E2 (PGE2) production (Moon and Pestka, 2002). In Jurkat T cells, DON induced phosphatidylserine externalization, mitochondrial release of cytochome c, procaspase-3 degradation, and Bcl-2 degradation (Nasri et al., 2006 [PMID:16472964]).

Recent studies have shown that DON-induced inhibition of cellular proliferation may occur through arrest of the cell cycle at G2/M and increased expression of cyclin related proteins (Xing et al., 2007; Yang et al., 2008 [PMID:18006205]).

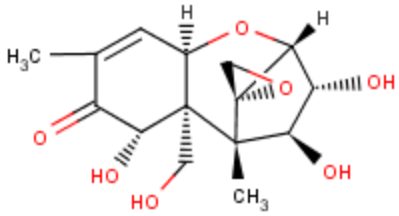
DON stimulated *in vitro* estradiol, but not progesterone synthesis, in porcine granulosa cells at concentrations <0.03 µM [9 ng/mL]. In combination with T2, it biphasically modulated progesterone synthesis. At a concentration of 0.01 µM [3 ng/mL], DON increased T2-toxin-induced stimulation of progesterone synthesis observed at a low concentration (0.0006 µM [0.2 ng/mL) and inhibited its effects reported at a high concentration (0.002 µM [0.6 ng/mL]) (Ranzenigo et al., 2006 abstr.).

DON modulated hepatic biotransformation enzymes in mice subchronically exposed to DON by oral administration (0.014-1.774 mg/kg [0.047-5.987 µmol/kg] bw). DON also increased expression of P4502b and cytostolic glutathione *S*-transferase π and α subfamilies (Gouze et al., 2006 [PMID:16209902]).

Evaluation of rodent feed for the presence of mycotoxins, including DON, showed that detectable levels were present in several samples evaluated. [ILS note: The potential presence of DON in animal feed could impact results and data interpretation] (Waldemarson et al., 2005 [PMID:15901367]).

## 10.0 Structure-Activity Relationships

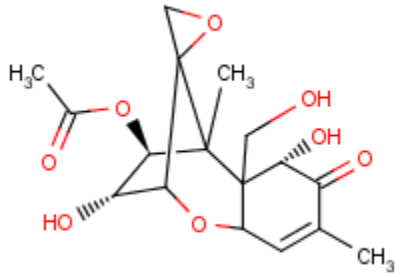
Nivalenol (NIV) [CAS No. 23282-20-4]




Like DON, NIV belongs to the type B-trichothecenes. It is one of the least acutely toxic trichothecenes; an oral $LD_{50}$ value of 39 mg/kg was reported for mice (Biopure, 2004). Distribution studies in pregnant and lactating mice showed that NIV is present in fetal and suckling mice tissues as well as the milk (Poapolathep et al., 2004 [PMID:15225570]). The absorption of NIV is rapid and excretion is mainly via feces. The major effects of subchronic/short-term and chronic toxicity experiments in mice were reduced body weight gain, reduced feed efficiency, changes in organ weight, and hematotoxicity (e.g., bone marrow toxicity). NIV was embryotoxic and fetotoxic but not teratogenic in the animals. A two-year feeding study in female mice did not produce tumors. The IARC concluded there "there is inadequate evidence in experimental animals for the carcinogenicity of nivalenol" and put it in Group 3 (Pronk et al., 2002). However, in a recent study, intermittent administration of NIV (5 µg) with 12-tetradecanoylphorbol-13-acetate induced papillomas and carcinomas in Balb/C mice (Hsia et al., 2004 [PMID:15254715]). NIV weakly induced chromosomal aberrations in mammalian cells *in vitro*. It can be immunosuppressive as well as immunostimulatory (Pronk et al., 2002).

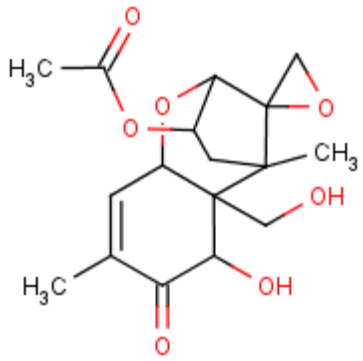
More recent studies support these early findings. In a one-month oral study using C57BL6 mice, an NOAEL of 1.774 mg/kg bw (equivalent to exposure to 5 ppm contaminated food) was determined for NIV; observations included increases in plasma phosphatase and IgG and decreases in plasma urine, IgM, and methoxyresorufin and pentoxyresorufin *O*-dealkylase activities at 8.87 mg/kg bw. At this dose, hepatotoxicity also was seen (Gouze et al., 2005 [PMID:16375817]). In a 90-day study using F344 rats, the NOAEL was established at <0.4 mg/kg bw/day (6.25 ppm) in both sexes; this was based on a decrease of white blood cell counts (at 100 ppm in males and 6.25 ppm in females), platelet counts (both sexes), red blood cell counts (males), and hemoglobin concentration (females) (all at 100 ppm). In addition, immune function in male rats was affected by NIV (Kubosaki et al., 2008 [PMID:17881110]; Takahashi et al., 2008 [PMID:17765382]). In a recent genotoxic study, NIV was reported to dose-dependently induce DNA damage in post-confluent human enterocyte-like Caco-2 cells (Bony et al., 2007 [PMID:17161579]).

Fusarenon X (FusX) [CAS No. 23255-69-8]



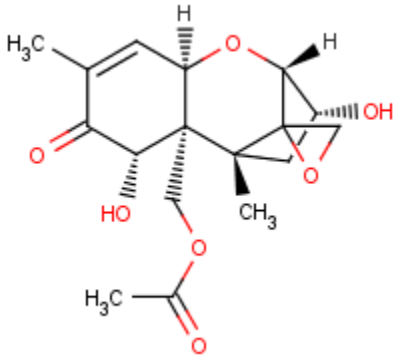

FusX is rapidly absorbed and deacetylated to NIV and excreted (Pronk et al., 2002). Distribution studies in pregnant and lactating mice showed that FusX is present in fetal and suckling mice tissues as well as the milk and maternal tissues; transfer of the compound to fetal or suckling mice occurred after metabolism to NIV (Poapolathep et al., 2004 [PMID:15225570]). The oral $LD_{50}$ values were 4.5 and 4.4 mg/kg bw in mice and rats. In short-term/subchronic and chronic studies, intrahepatic bile duct hyperplasia, atypical hyperplasia in the gastric and intestinal mucosa, and hypoplasia and atrophy of bone marrow, thymus, and spleen were reported. FusX was embryotoxic and fetotoxic but not teratogenic in mice; it inhibited pregnancy, retarded fetal growth, and caused fetal death. The IARC concluded that "there is inadequate evidence in experimental animals for the carcinogenicity of fusarenon X" and put it in Group 3. Although it failed to induce gene mutations in bacteria and mammalian cells, it produced some chromosome breaks in mammalian cells. FusX is also immunosuppressive and cytotoxic (Pronk et al., 2002). In a more recent genotoxic study, FusX was reported to dose-dependently induce DNA damage in post-confluent human enterocyte-like Caco-2 cells and increase DNA strand breaks in dividing cells (Bony et al., 2007 [PMID:17161579]). Additionally, in a more recent cytotoxic study, FusX was apoptotic in Jerkat human T cells, causing 50% cell death at a concentration of 7.5 µg/mL. It suppressed IL-2 upregulation (62.5-500 ng/mL) but had no effect on IL-8 production (Pestka et al., 2005 [PMID:15588914]).

3-Acetyldeoxynivalenol (3-Ac-DON) [CAS No. 50722-38-8]]



3-Ac-DON is deacetylated to DON by rabbit liver carboxy esterases. $LD_{50}$ values (i.p., 49-54 mg/kg bw; subcutaneous [s.c.], 59 mg/kg bw; oral 34 mg/kg bw) in mice indicate it to be acutely toxic. The main effects of an acute toxicity study with 3-Ac-DON in mice were on dividing cells of the duodenal crypts, thymus, and spleen. In short-term/subchronic studies in mice, feed consumption and body weights were reduced during the first few weeks of treatment, eventually returning to normal. Liver weight was increased in a 48-day feeding trial with 3-Ac-DON (20 mg/kg feed). 3-Ac-DON was not mutagenic in the Ames test but did induce chromosomal aberrations in Chinese hamster V79 cells *in vitro*. It is immunosuppressive and immunostimulating, as well as cytotoxic to cultured cells (Pronk et al., 2002). In Jerkat human T cells, 3-Ac-DON was apoptotic causing 50% cell death at a concentration of 0.5 µg/mL. It upregulated IL-8 production (625-5000 ng/mL) but had no effect on IL-2 (62.5-500 ng/mL) (Pestka et al., 2005 [PMID:15588914]).

15-Acetyldeoxynivalenol (15-Ac-DON) [CAS No. 88337-96-6]



15-Ac-DON is acutely toxic, with oral and i.p. $LD_{50}$ values of 34 and 113 mg/kg bw, respectively, in mice. It mainly affects the dividing cells of bone marrow, thymus, spleen, and intestines. 15-Ac-DON also produced focal lesions in the kidneys of mice. In short-term/subchronic studies, feed consumption and body weight gain were reduced in mice. Additionally, absolute liver, kidney, and spleen weights were decreased, while relative spleen and kidney weights were increased. 15-Ac-DON is immunosuppressive and immunostimulating. It inhibited and superinduced cytokine production and mRNA expression in murine spleen CD4+ T cells (Pronk et al., 2002). In Jerkat human T cells, 15-Ac-DON was apoptotic, causing 50% cell

death at a concentration of 0.5 µg/mL. It upregulated IL-8 production but had no effect on IL-2 at doses ranging from 62.5-500 ng/mL (Pestka et al., 2005 [PMID:15588914]).

## 11.0 Comparative Toxicity with Other Trichothecenes and Related *Fusarium* Mycotoxins

There are a number of mycotoxins that may occur simultaneously in grain and feed sources, however synergism and antagonism between different combinations of these contaminants has not been thoroughly studied. DON and NIV, which are both type B trichothecenes, are often found together in the same samples of grain or animal feed. Other type B (e.g., FusX) and/or type A (e.g., T-2 toxin or diacetoxyscirpenol) trichothecenes or *Fusarium*-produced mycotoxins (e.g., zearalenone, fumonisin $B_1$, or fusarin C) might also occur in combination with DON and/or NIV. The type of mycotoxins present and the extent to which they are produced depends on the grain source, the *Fusarium* species present, and the climate conditions. For example, the fumonisins and fusarin C occur primarily on maize, particularly when it is grown under warm, dry conditions.

The type A trichothecenes tend to be present in lower quantities than the type B but are more toxic. DON has been reported to be the least toxic type B and T-2 the most toxic type A of the commonly detected trichothecenes. It is thought that both types of trichothecenes have the same mechanism of action in the body and therefore would produce an additive effect. There is also evidence that suggest that mycotoxins may have synergistic effects with other fungal metabolites, metabolites originating from host plants, or compounds added to grain or feed sources. Studies have shown that pure zearalenone is less toxic than cereals naturally contaminated with zearalenone, indicating the presence of additional toxic substances in the matrix. Whether these additional substances are metabolites of molds or are components in food or feed due to pesticide residues or food additives is not clear.

In a yeast (*Kluyveromyces marxianus*) bioassay, DON plus NIV had a synergistic response on the toxicity, while DON plus T-2 toxin had an antagonistic effect. In mouse fibroblast L929 cells, a mixture containing DON, NIV, T-2 toxin, zearalenone, and fumonisin $B_1$ produced greater inhibition of DNA synthesis than with treatment of each mycotoxin alone.

Results from toxicological studies of two type A trichothecenes (diacetoxyscirpenol and T-2 toxin), three type B trichothecenes (DON, NIV, and FusX), and three additional *Fusarium*-produced mycotoxins (zearalenone, fumonisin $B_1$, and fusarin C) are summarized in this section. The qualitative results from studies of acute and short-term toxicity, reproductive toxicity, developmental toxicity, immunotoxicity, genotoxicity, and carcinogenicity in rats, mice, and, in some cases, humans are presented in **Table 3**. A brief description of the study results for each agent is provided based on the endpoints included in the table. All of the information presented here was abstracted from authoritative reviews and/or reports (Eskola, 2002; IARC, 1993, 2002; JECFA, 2001b, 2001c; NTP, 1982, 2001; Pronk et al., 2002; WHO, 2000).

## Table 3. Comparative Toxicity

| Agent | CASRN | Toxicity - $LD_{50}$ (mg/kg bw) | | Repro/Develop Toxicity | | Immunotoxicity | | Genotoxicity | | | | Carcinogenicity | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mm | Rn | Mm | Rn | Mm | Rn | Sal | DD | GM | CG | Hm | Mm | Rn |
| ***Trichothecenes - Type B*** | | | | | | | | | | | | | | |
| Deoxynivalenol | 51481-10-8 | 78 | n.p. | R–, D+ | R–, D– | + | + | – | – | – | + | I | I | I |
| Nivalenol | 23282-20-4 | 38.9 | 19.5 | R+, D– | n.p. | + | n.p. | n.p. | n.p. | n.p. | (+) | I | I | I |
| Fusarenon X | 23255-69-8 | 4.5 | 4.4 | R+, D– | n.p. | + | n.p. | – | + | – | + | I | I | I |
| ***Trichothecenes - Type A*** | | | | | | | | | | | | | | |
| Diacetoxyscirpenol | 2270-40-8 | 15.5 | 7.5 | D+ | R– | + | n.p. | – | n.p. | n.p. | + | | | |
| T-2 Toxin | 21259-20-1 | 10 (5.2)[a] | (1.3)[a] | D+ | n.p | + | + | – | + | + | + | I | L | L |
| ***Other Fusarium mycotoxins*** | | | | | | | | | | | | | | |
| Zearalenone | 17924-92-4 | 2000 | 4000 | R+, D– | R+, D– | – | n.p. | – | n.p. | – | + | I | L | L |
| Fumonisin $B_1$ | 116355-83-0 | n.p. | n.p. | D+ | D+ | n.p. | n.p. | – | + | n.p. | + | I | S | S |
| Fusarin C | 79748-81-5 | n.p. | n.p. | n.p. | n.p. | n.p. | n.p. | + | + | + | + | I | L | L |

[a] Intraperitoneal

Abbreviations: CG = cytogenetic damage (including sister chromatid exchange); D = Developmental toxicity (e.g., fetal malformation); DD = DNA damage (e.g., strand breaks, unscheduled DNA synthesis); GM = mammalian cell gene mutation; Hm = Human; I = Inadequate evidence; L = Limited evidence or some evidence; Mm = Mouse; n.p. = not provided; Rn = Rat; R = Reproductive toxicity; S = Sufficient evidence or clear evidence; Sal = Salmonella; + = positive; (+) = weakly positive; – = negative

## 11.1 Toxicity

The oral $LD_{50}$ values obtained for mice and rats are presented in **Table 3**. The toxic effects reported for many of the mycotoxins summarized here include reduced body weight gain, hematotoxicity, neurotoxicity, hemorrhages, skin irritation, vomiting, and/or diarrhea. It has been reported that DON was a contributing factor to the acute outbreaks of red mold toxicosis that involved *F. graminearum* and affected thousands of people in China, India, and Japan in the 1980s.

***DON***

Feed intake and body weight gain were significantly decreased in male mice fed a diet containing DON for 12 weeks. Blood insulin, glucose, and free fatty acids increased in male rats receiving s.c. injections of DON for three days. A statistically significant increase in glycogen and reduction in triglycerides content in muscle tissue was also observed. Similar exposure via i.p. infusion produced comparable effects.

***NIV***

The primary toxic effects reported for subacute and subchronic toxicity experiments in mice exposed to NIV were reduced body weight gain and reduced feed efficiency, organ weight changes (no histopathology findings) and bone marrow toxicity, leukopenia and/or erythrocytopenia, depending on the length of the study. In subacute feeding studies with pigs, NIV caused mild pathological changes in kidney, spleen, and gastrointestinal tract. Leukocyte counts, body weight gain and food consumption were not affected.

***FusX***

FusX is very toxic by acute oral administration in mice and rats as noted by the $LD_{50}$ values of 4.5 and 4.4 mg/kg bw, respectively. Only a few repeat dose studies have been reported. In these studies, FusX caused intrahepatic bile duct hyperplasia, atypical hyperplasia in the gastric and intestinal mucosa and hypoplasia and atrophy of the bone marrow, thymus, and spleen of male rats.

***Diacetoxyscirpenol***

Rats have been reported to be more sensitive than mice to systemic effects of diacetoxyscirpenol (DAS) applied topically, but mice were more sensitive to skin damage. The primary toxic effects observed in acute and in limited subacute studies (considered only a few effects, and some only used one dose) were similar consisting of hematological changes (leukopenia and/or anemia), cell depletion and necrosis of the lympho-hematopoietic organs (bone marrow, thymus, spleen, lymph nodes) and intestines and degeneration of testes.

***T-2 Toxin***

Studies of adverse health effects in humans associated with outbreaks of acute poisoning reported that analyses of limited numbers of suspected grain or food samples indirectly linked T-2 toxin the outbreaks. Some of the symptoms reported included nausea, vomiting, pharyngeal irritation, abdominal pain and distension, diarrhea, bloody stools, dizziness, and chills. In one of the outbreaks other trichothecenes were reported to be present with T-2 toxin, including deoxynivalenol, acetyldeoxy-NIV, and NIV. The presence of these or other contaminants could not therefore be ruled out in other studies.

Molds implicated in the outbreak of a series of food-related poisoning referred to as alimentary toxic aleukia in the former Soviet Union in 1931-1947 were shown to be able to produce T-2 toxin when cultured three decades after the outbreak. The main pathological changes reported among victims were necrotic lesions of the oral cavity, esophagus, and stomach and, in particular, pronounced leukopenia consisting primarily of bone-marrow hypoplasia and aplasia.

T-2 toxin fed to rats for 4 weeks caused gastric lesions which were diffuse and severe in the rats at the highest dose and focal but definite in rats receiving a lower dose. Rats that received T-2 toxin via s.c. injection daily for 28 days showed changes in the liver, kidney, and heart ranging from dystrophy or necrosis to hyperplasia.

***Zearalenone***

In male mice receiving 1000 or 3000 mg/kg zearalenone in the diet for 13 weeks, atrophy of the seminal vesicles and testes and cytoplasmic vacuolization of the adrenals were reported. Squamous metaplasia of the prostate was also seen at the highest dose. In female mice, endometrial hyperplasia of the uterus was reported for all dose groups (30-3000 mg/kg of diet; incidence was not dose-related). Osteoporosis was observed in males and females at doses as low as 100 mg/kg, and myelofibrosis of the bone marrow was seen at doses ≥300 mg/kg. Weight gain was reduced 14% or more at doses ≥300 mg/kg of diet and two females fed 3000 mg/kg of diet died.

Zearalenone given to weanling female mice in the diet at 10 mg/kg of diet for eight weeks did not alter body weight gain or feed intake. No histopathological changes were seen in the thymus, spleen, liver, kidney, uterus, small intestine, colon, heart, brain, lungs, and bone marrow. A significant increase ($p<0.01$) was seen in the number of erythrocytes in treated animals but other hematological parameters were unchanged.

In male rats receiving 1000 or 3000 mg/kg zearalenone in the diet for 13 weeks, atrophy of the seminal vesicles and fibromuscular hyperplasia of the prostate were observed. Ductular hyperplasia of the mammary gland and hyperplasia of the pituitary was also seen in males and females at these doses. In female rats fed ≥100 mg/kg of diet, endometrial hyperplasia of the uterus was reported. Osteoporosis was observed in males at the two highest doses and in all treated females. Weight gain was reduced 17% or more at dietary concentrations ≥100 mg/kg and no treatment-related deaths occurred.

***Fumonisin $B_1$***

In all animal species tested, fumonisin $B_1$ has been shown to be hepatotoxic and nephrotoxic. The initial changes reported to occur in both liver and kidney are increased apoptosis followed by regenerative cell proliferation. Fumonisin $B_1$ has also been reported to cause equine leukoencephalomalacia and porcine pulmonary edema syndrome in domestic animals. Although its acute toxicity is low, these diseases occur with rapid onset and both involve interference with sphingolipid metabolism and cardiovascular dysfunction.

***Fusarin C***

Fusarin C inhibited incorporation of valine into proteins in rat hepatocyte cultures and cellular growth in lymphoma cells *in vitro*. Results from studies of macrophages treated *in vitro* suggest that fusarin C is generally not toxic to the cells. No data were available from *in vivo* studies.

## 11.2 Reproductive and Developmental Toxicity

***DON***

In three-month-old pregnant mice, i.p. injection of DON produced high maternal deaths at the two highest doses. The number of resorptions was dose-dependently increased. Skeletal abnormalities (mostly in the axial skeleton) were also observed in the fetuses. Exencephaly was mainly seen at the mid-doses during the four-day treatment. At the higher dose and shorter exposure period, neural arch defects or fusion were primarily seen.

***NIV***

Stillbirths preceded by vaginal hemorrhage were observed in pregnant mice injected i.p. with NIV on days 7-15 of gestation. A high incidence of embryolethality was recorded in the two highest dose groups but no fetal malformations were observed in any of treated animals. A single injection of a relatively high dose of NIV on day 7 affected the embryo within 10 hours, damaged the placenta within 24 hours, and caused stillbirths at 48 hours. Embryotoxicity associated with maternal weight loss was reported for mice fed diets with moldy rice powder containing NIV or administered purified NIV by gavage on days 7-15 of gestation. These effects were seen at the highest nonlethal doses. Intrauterine growth retardation in the term fetuses from all exposure groups was also reported. No significant adverse effects were reported for gross, skeletal, and visceral malformations.

***FusX***

Pregnant mice were injected s.c. with FusX either at a single dose on day 6, 8, 10 or 13 of gestation, or at multiple doses on days 8-12 or 8-14 of gestation. Dams given the highest dose (4.1 mg/kg bw) died within 24 hours of injection; dams receiving the second highest dose (2.6 mg/kg bw) aborted one day after injection. Abortion occurred less frequently and at longer intervals after injection with doses of 1.6 mg/kg bw and below. Embryotoxicity was reported to be dose-dependent based on the number of resorbed and dead fetuses. Multiple doses caused all animals to abort at the mid- and high-dose levels, but not at the lowest dose level (0.63 mg/kg bw). FusX inhibited embryonal implantation in pregnant mice fed diets mixed with FusX throughout gestation or during early pregnancy. It also induced abortion, fetal absorption, and fetal growth retardation in mice fed FusX throughout gestation or at early, mid or late pregnancy. No teratogenic effects were reported.

***DAS***

DAS given to pregnant mice by single i.p. injection on one of gestation days 7-11 induced maternal toxicity (death and vaginal bleeding) at the two highest doses (3 and 6 mg/kg bw). Although no effect on implantation was reported, resorptions increased with dose and with day of injection. Fetal body weight was significantly depressed at all doses and fetal malformations observed included exencephaly, omphalocele, hydrocephaly, short snout, protruding tongue and meningoencephalocele and skeletal anomalies of the skull, sternebrae, vertebrae, vertebral centra, and ribs, especially when DAS was given on day 9 of gestation.

In male rats DAS reduced testicular weight and sperm production after a single i.p. injection. The frequency of hypocellular seminiferous tubules was also increased and the tubules had few or no germinal epithelial cells (almost entirely vacuolated Sertoli cells). DAS was also reported to cause alterations in epididymal transit times, as indicated by decreased epididymal sperm reserves.

***T-2 Toxin***

Intraperitoneal injections of T-2 toxin into pregnant mice on days 7-11 resulted in significant decrease in fetal weight, reduction in litter sizes, and gross malformations. The malformations included missing tails, deformed limbs, exencephaly, retarded jaw development, and open eyes.

***Zearalenone***

Eight months after treatment of newborn female mice with zearalenone by daily s.c. injection for five days, 70% of the animals were reported to have no corpora lutea and many had dense collagen deposition in the uterine stroma. In addition, 56% had no uterine glands, and 59% had squamous metaplasia. Zearalenone caused persistent estrus in 60-80% of animals, delay in vaginal opening, and sterility along with thickening of the vaginal epithelium at eight weeks of age in female mice treated on days 1-3 or 1-5 after birth. No teratogenic effects in mice were reported.

After receiving zearalenone in the diet for four weeks, $F_0$ male rats were mated with non-treated females to produce an $F_{1a}$ generation. The $F_{1a}$ generation was bred at sexual maturity to give an $F_{2a}$ generation. The $F_0$ and $F_{1a}$ generations were given a zearalenone-containing diet throughout mating and gestation. The number of live-born $F_1$ pups per litter was reduced only at the high dose (10 mg/kg bw per day) but the number of live-born $F_2$ pups per litter was also reduced at the low dose (1 mg/kg bw). Fertility was significantly decreased at the high dose in both the $F_1$ and $F_2$ generations. Zearalenone did not affect the rate of survival of live-born pups up to 4 or 21 days of age. No significant developmental effects were reported in rats.

***Fumonisin $B_1$***

Fumonisin $B_1$ has been reported to cause developmental toxicity in several animal species, including rats, mice and rabbits. Developmental effects occurred at dose levels that caused maternal toxicity in liver and kidney and were associated with disruption of sphingolipid metabolism. Survival of rat pups decreased after postnatal exposure to fumonisin $B_1$.

***Fusarin C***

No data were available.

### 11.3 Immunotoxicity

***DON***

See Section 9.9 of this report.

***NIV***

Both antiviral and immunotoxic activity have been reported for NIV. Depending on dose and exposure regimen, this activity may be immunosuppressive or immunostimulatory. Given in the

diet at concentrations of 6 or 12 mg/kg for 4 or 8 weeks, NIV elevated IgA deposits in the glomerular mesangium of mice in a time and dose-dependent fashion. The pathological changes observed resembled those in human IgA nephropathy.

*In vitro*, NIV inhibited blastogenesis in cultured human lymphocytes, proliferation and immunoglobulin production (IgA, IgG and IgM) in mitogen-stimulated human lymphocytes, and T and B cell proliferation in human peripheral blood mononuclear cells. NIV also suppressed natural killer cell activity and inhibited antibody-dependent cytotoxicity in mononuclear cells. When cells were exposed to low levels of NIV, immunoglobulin production (especially IgA) was elevated and cell proliferation increased.

***FusX***

In mice given daily i.p. treatment with FusX an immunosuppressive effect was reported. IgE and IgG1 antibody formation was suppressed *in vivo*, and antibody formation by splenic lymphocytes raised by T-dependent and independent mitogens was suppressed *in vitro*. FusX also inhibited blastogenesis in cultured human lymphocytes and T and B cell proliferation in human peripheral blood mononuclear cells *in vitro*.

***DAS***

As with NIV, DAS may exhibit immunosuppressive or immunostimulatory activity depending on dose and exposure regimen. In mice treated with DAS by i.p. injection, thymus weights were reduced and the responsiveness to sheep red blood cells (SRBC; inhibition) was suppressed.

*In vitro*, DAS inhibited T and B cell stimulation and the ability of mitogen-stimulated murine splenic and thymic lymphocytes to synthesize anti-SRBC antibodies. This activity was reversible. DAS inhibited immunoglobulin production (IgA, IgG and IgM) and proliferation of mitogen-stimulated human lymphocytes. At low concentrations, however, DAS demonstrated immunostimulatory activity by enhancing proliferative responses and elevating immunoglobulin production (especially IgA).

***T-2 Toxin***

Apoptosis was induced in thymus and spleen of mice given T-2 toxin by oral gavage. T-2 toxin caused specific depletion of myelocytes in mouse bone marrow and reversibly inhibited stimulation of both T and B cells. The ability to synthesize antibodies to sheep red blood cells was also suppressed. T-2 toxin was reported to decrease resistance to infection in both mice and rats.

***Zearalenone***

Mice fed a diet supplemented with zearalenone (10 mg/kg of diet) for two weeks had an increase in splenic bacterial count 1 and 4 days after intravenous injection with *Listeria monocytogenes* cells. No adverse effects were observed after eight weeks of feeding including responsiveness to sheep red blood cells and delayed hypersensitivity response to keyhole hemocyanin. Zearalenone given to female mice by s.c. injection had no effect on survival rate or splenic bacterial count following infection with *L. monocytogenes* cells. Similarly, zearalenone given to weanling female mice in the diet (10 mg/kg of diet) for six weeks had no effect on

immunoglobulins G, M, or A concentrations in the serum or on the leukocyte count or differential lymphocyte, polymorphonuclear neutrophil, monocyte, or eosinophil counts.

***Fumonisin $B_1$***
No data were available.

***Fusarin C***
No data were available.

### 11.4 Genotoxicity

***DON***
In cultured mammalian cells, DON induced cell transformation, chromosomal aberrations, and inhibition of gap-junctional intercellular communication. It did not induce unscheduled DNA synthesis or mutation in cultured mammalian cells or *S. typhimurium*.

***NIV***
Results from two studies reported that NIV induced chromosomal aberrations in Chinese hamster V79 cells *in vitro* and, to a lesser degree, sister chromatid exchanges.

***FusX***
Limited data from three studies showed FusX induced chromosomal aberrations and sister chromatid exchanges in Chinese hamster V79 cells and DNA single-strand breaks in human HeLa cells *in vitro*. It was not mutagenic to mouse mammary carcinoma cells *in vitro* or *S. typhimurium*.

***DAS***
In male Swiss mice exposed by i.p. injection, DAS induced chromosomal aberrations in bone marrow and in germ cells (spermatocytes). It also induced an increase in sperm head abnormalities (especially amorphous and small heads) and tail abnormalities (coiled tails). DAS did not induce sister chromatid exchanges in human lymphocytes *in vitro* and was not mutagenic to *S. typhimurium*, with and without metabolic activation.

***T-2 Toxin***
T-2 Toxin induced DNA damage and chromosomal aberrations *in vivo*, and in cultured human and rodent cells. It also induced chromosomal aberrations in Drosophila and gene mutation in cultured rodent cells. T-2 toxin was not mutagenic to *S. typhimurium* and did not induce DNA damage in bacteria.

***Zearalenone***
In cultured rodent cells zearalenone induced chromosomal anomalies. It did not induce recombination in yeast or gene mutation or DNA damage in *S. typhimurium*.

***Fumonisin $B_1$***
Fumonisin $B_1$ induced micronuclei in mouse bone marrow and DNA fragmentation in rat liver and spleen *in vivo*. It also caused DNA damage, chromosomal aberrations, and micronuclei in

cultured mammalian cells but did not induce unscheduled DNA synthesis in rat hepatocytes and was not mutagenic to *S. typhimurium*.

***Fusarin C***
Fusarin C induced chromosomal aberrations, gene mutation, and DNA damage in cultured mammalian cells in single studies. It also induced mutations in *S. typhimurium*.

## 11.5 Carcinogenicity

***DON***
See Section 9.3 of this report.

***NIV***
No increase in tumor incidence was reported in a single study of female mice given NIV by oral administration in the diet.

***FusX***
FusX was tested for carcinogenicity in two studies in male rats by oral administration and in male mice and male rats by s.c. injection. The studies were inadequate for evaluation.

***DAS***
Results from a single skin painting study showed that DAS applied to the skin of the back of mice twice a week, for one year did not induce any skin tumors. Necrosis of the skin was noted.

***T-2 Toxin***
T-2 toxin given to mice by oral administration in the diet increased the incidence of pulmonary and hepatic adenomas in males. Results from studies of rats given T-2 toxin by intragastric administration were inadequate for evaluation.

***Zearalenone***
Zearalenone administration in the diet to mice in one experiment and rats in two experiments caused an increase in the incidence of hepatocellular adenomas in female mice and of pituitary adenomas in mice of each sex. No increase in tumor incidence was observed in rats.

***Fumonisin $B_1$***
Fumonisin $B_1$ given by oral administration in the diet in one experiment to male rats, induced hepatocellular carcinomas. It also induced the formation of foci of altered (gamma-glumatyltranspeptidase-positive) hepatocytes. Clear evidence of carcinogenic activity of fumonisin $B_1$ in male, but not female, F344/N rats was also reported by NTP based on the increased incidences of renal tubule neoplasms. Clear evidence of carcinogenic activity in female, but not male, $B6C3F_1$ mice was also reported based on the increased incidences of hepatocellular neoplasms.

A culture of *F. moniliforme* known to produce significant amounts of fumonisins $B_1$ and $B_2$ were given orally to male rats in two experiments. An increased incidence of neoplastic nodules, hepatocellular carcinomas and cholangiocellular carcinomas was reported. In addition,

forestomach papillomas and carcinomas were observed. Two studies in which male rats were fed maize naturally contaminated with *F. moniliforme* were inadequate for evaluation.

***Fusarin C***

Fusarin was tested in one study in female mice and female rats by oral gavage. It induced papillomas and carcinomas of the esophagus and forestomach in both mice and rats. Oral exposure of male rats to a culture of *F. moniliforme* known to contain mainly fusarin C did not induce tumors.

## 12.0 Online Databases and Secondary References

### 12.1 Online Databases

National Library of Medicine Databases (TOXNET)

| | |
|---|---|
| ChemIDplus | Household Products |
| CCRIS | HSDB |
| DART | IRIS |
| EMIC and EMICBACK | TOXLINE |
| GENETOX | TRI |

STN International Files

| | |
|---|---|
| AGRICOLA | IPA |
| BIOSIS | MEDLINE |
| BIOTECHNO | NIOSHTIC |
| CABA | NTIS |
| CANCERLIT | Registry |
| EMBASE | RTECS |
| ESBIOBASE | TOXCENTER |

TOXCENTER includes toxicology data from the following files:

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|---|---|
| Aneuploidy | ANEUPL* |
| BIOSIS Previews® (1969-present) | BIOSIS* |
| CAplus (1907-present) | CAplus |
| International Labour Office | CIS* |
| Toxicology Research Projects | CRISP* |
| Development and Reproductive Toxicology | DART®* |
| Environmental Mutagen Information Center File | EMIC* |
| Epidemiology Information System | EPIDEM* |
| Environmental Teratology Information Center File | ETIC* |
| Federal Research in Progress | FEDRIP* |
| Health Aspects of Pesticides Abstract Bulletin | HAPAB |
| Hazardous Materials Technical Center | HMTC* |
| International Pharmaceutical Abstracts (1970-present) | IPA* |
| MEDLINE (1951-present) | MEDLINE |
| Pesticides Abstracts | PESTAB* |
| Poisonous Plants Bibliography | PPBIB* |
| Swedish National Chemicals Inspectorate | RISKLINE |

TOXCENTER includes toxicology data from the following files:

| | |
|---|---|
| Toxic Substances Control Act Test Submissions | TSCATS* |

*These are also in TOXLINE. Missing are TOXBIB, NIOSHTIC®, NTIS.

In-House Databases
Current Contents on Diskette®
The Merck Index, 1996, on CD-ROM

## 12.2 Secondary References

Budavari, S., Ed. 1996. The Merck Index, 12th ed. Merck and Company, Inc., Whitehouse Station, NJ. CD-ROM version 12:1 1996, Chapman & Hall Electronic Publishing Division.

## 13.0 References

Abbas, H.K., Mirocha, C.J., Pawlosky, R.J., and Pusch, D.J. 1985. Effect of cleaning, milling and baking on deoxynivalenol in wheat. Appl Environ Microbiol, 50(2):482-486. Internet address: http://www.pubmedcentral.nih.gov/articlerender.fcgi?tool=pubmed&pubmedid=4051489. Last accessed on July 8, 2008.

Alexander, N.J. 2008. The TRI101 story: engineering wheat and barley to resist *Fusarium* head blight. World Mycotoxin J, 1(1):31-37. Abstract from CABA 2008:245014.

Alm, H., Greising, T., Brussow, K.-P., Torner, H., and Tiemann, U. 2002. The influence of the mycotoxins deoxynivalenol and zearalenol on *in vitro* maturation of pig oocytes and *in vitro* culture of pig zygotes. Toxicol *in Vitro*, 16(6):643-648. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/12423645?ordinalpos=2&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 9, 2008.

Amuzie, C.J., Harkema, J.R., and Pestka, J.J. 2008. Tissue distribution and proinflammatory cytokine induction by the trichothecene deoxynivalenol in the mouse: comparison of nasal vs. oral exposure. Toxicology, 248(1):39-44. Abstract from PubMed 18433975. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=18433975. Last accessed on July 7, 2008.

Anaya, I., Ventura, M., Agut, M., and Cornellas, L. 2004. Rapid screening method for deoxynivalenol, T-2 toxin and diacetoxyscirpenol in maize-based foods by thin-layer chromatography. Afinidad, 61(510):124-128. Abstract from TOXCENTER 2004:176266.

Aziz, N.H., Attia, E.S., and Farag, S.A. 1997. Effect of gamma-irradiation on the natural occurrence of *Fusarium* mycotoxins in wheat, flour, and bread. Nahrung, 41(1):34-7. Abstract from PubMed 9113669. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/9113669?ordinalpos=7&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on June 26, 2008.

Bae, H.K., and Pestka, J.J. 2008. Deoxynivalenol induces p38 interaction with the ribosome in monocytes and macrophages. Toxicol Sci [Epublication ahead of print]. Abstract from PubMed 18502741. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=18502741. Last accessed on July 8, 2008.

Baltriukiene, D., Kalvelyte, A., and Bukelskiene, V. 2007. Induction of apoptosis and activation of JNK and p38 MAPK pathways in deoxynivalenol-treated cell lines. Altern Lab Anim, 35(1):53-59. Abstract from PubMed 17411352. PubMed abstract Internet address:

http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=17411352. Last accessed on July 7, 2008.

Berek, L., Petri, I.B., Mesterházy, Á., Téren, J., and Molnár, J. 2001. Effects of mycotoxins on human immune functions *in vitro*. Toxicol *in Vitro*, 15(1):25-30. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/11259866?ordinalpos=2&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 9, 2008.

Berthiller, F., Dall'Asta, C., Schuhmacher, R., Lemmens, M., Adam, G., and Krska, R. 2005. Masked mycotoxins: determination of a deoxynivalenol glucoside in artificially and naturally contaminated wheat by liquid chromatography-tandem mass spectrometry. J Agric Food Chem, 53(9):3421-3425. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=15853382. Last accessed on March 6, 2009.

Bretz, M., Beyer, M., Cramer, B., Knecht, A., and Humpf, H.U. 2006. Thermal degradation of the *Fusarium* mycotoxin deoxynivalenol. J Agric Food Chem, 54(17):6445-51. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=16910743. Last accessed on March 5, 2009.

Bily, A.C., Reid, L.M., Savard, M.E., Reddy, R., Blackwell, B.A., Campbell, C.M., Krantis, A., Durst, T., Philogene, B.J., Arnason, J.T., and Regnault-Roger, C. 2004. Analysis of *Fusarium graminearum* mycotoxins in different biological matrices by LC/MS. Mycopathologia, 157(1):117-26. Abstract from PubMed 15008354. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/15008354?ordinalpos=2&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on June 26, 2008.

Biopure. 2004. Mycotoxin data collection: Nivalenol. Internet address: http://www.biopure.at/biopure-index/datasheets/mdc/DataCollection_Mycs/Nivalenol.pdf. Last accessed on June 25, 2008.

Birzele, B., Meier, A., Hindorf, H., Kramer, J., and Dehne, H.W. 2002. Epidemiology of *Fusarium* infection and deoxynivalenol content in winter wheat in the Rhineland, Germany. Eur J Plant Pathol, 108(7):667-673. Abstract from AGRICOLA 2003:36586.

Bony, S., Carcelen, M., Olivier, L., and Devaux, A. 2006. Genotoxicity assessment of deoxynivalenol in the Caco-2 cell line model using the Comet assay. Toxicol Lett, 166(1):67-76. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=16828243. Last accessed on July 7, 2008.

Bony, S., Olivier-Loiseau, L., Carcelen, M., and Devaux, A. 2007. Genotoxic potential associated with low levels of the *Fusarium* mycotoxins nivalenol and fusarenon X in a human intestinal cell line. Toxicol *In Vitro*, 21(3):457-465. Abstract from PubMed 17161579. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=17161579. Last accessed on July 7, 2008.

Bucheli, T.D., Erbs, M., Hartmann, N., Vogelgsang, S., Wettstein, F.E., and Forrer, H.-R. 2005. Estrogenic mycotoxins in the environment. Mitt Lebensmittelunters Hyg, 96(6):386-403. Abstract from TOXCENTER 2006:126257.

Bucheli, T.D., Wettstein, F.E., Hartmann, N., Erbs, M., Vogelgsang, S., Forrer, H.R., and Schwarzenbach, R.P. 2008. *Fusarium* mycotoxins: overlooked aquatic micropollutants? J Agric Food Chem, 56(3):1029-34. Abstract from PubMed 18197623. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=18197623. Last accessed on March 5, 2009.

Calvert, T.W., Aidoo, K.E., Candlish, A.G., and Fuat, A.R. 2005. Comparison of *in vitro* cytotoxicity of *Fusarium* mycotoxins,deoxynivalenol, T-2 toxin and zearalenone on selected human epithelial cell lines. Mycopathologia, 159(3):413-419. Abstract from PubMed 15883728. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=15883728. Last accessed on July 7, 2008.

Campbell, H., Choo, T.M., Vigier, B., and Underhill, L. 2002. Comparison of mycotoxin profiles among cereal samples from eastern Canada. Can J Botany, 80(5):526-532. Abstract from CABA 2002:162800.

Cetin, Y., and Bullerman, L.B. 2005. Cytotoxicity of *Fusarium* mycotoxins to mammalian cell cultures as determined by the MTT bioassay. Food Chem Toxicol, 43(3):755-764. Abstract from PubMed 15778016. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=15778016. Last accessed on July 7, 2008.

Chekowski, J., Stepien´, L., Tomczak, M., and Wiśniewska, H. 2002. Identification of toxicogenic *Fusarium* species in wheat using PCR assay and their mycotoxins in kernels. Phytopathol Polonica, 25:47-57. Abstract from CABA 2002:204766.

Chung, Y.-J., Zhou, H.-R., Pestka, J.J. 2003a. Transcriptional and posttranscriptional roles for p38 mitogen-activated protein kinase in upregulation of TNF-α expression by deoxynivalenol (vomitoxin). Toxicol Appl Pharmacol, 193(2):188-201. Abstract from PubMed 14644621. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/14644621?ordinalpos=8&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 3, 2008.

Chung, Y.J., Yang, G.H., Islam, Z., and Pestka , J.J. 2003b. Up-regulation of macrophage inflammatory protein-2 and complement 3A receptor by the trichothecenes deoxynivalenol and satratoxin G. Toxicology, 186(1-2):51-65. Abstract from PubMed 12604170. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/12604170?ordinalpos=6&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 3, 2008.

Cirillo, T., Ritieni, A., Galvano, F., and Amodio Cocchieri, R. 2003. Natural co-occurrence of deoxynivalenol and fumonisins B1 and B2 in Italian marketed foodstuffs. Food Addit Contam, 20(6):566-571. Abstract from PubMed 12881130. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/12881130?ordinalpos=2&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on June 26, 2008.

Clear, R.M., Patrick, S.K., and Gaba, D. 2000a. Prevalence of fungi and fusariotoxins on barley seed from western Canada, 1995 to 1997. Can J Plant Pathol, 22(1):44-50. Abstract from CABA 2000:81131.

Clear, R.M, Patrick, S.K., and Gaba, D. 2000b. Prevalence of fungi and fusariotoxins on seed from western Canada, 1995-1997. Can J Plant Pathol, 22(3):310-314. Abstract from AGRICOLA 2001:18780.

Codex Alimentarius Commission. 2007. Joint FAO/WHO Food Standards programme; Codex Committee on Contaminants in Foods; First Session; Beijing, China, April 16-20, 2007. Discussion paper on deoxynivalenol. CX/CF 07/1/17. Internet address: ftp://ftp.fao.org/codex/cccf1/cf01_17e.pdf. Last accessed on March 4, 2009.

Coffey, R., Cummins, E., and Ward, S. 2009. Exposure assessment of mycotoxins in dairy milk. Food Control, 20(3):239-249.

Collins, T.F.X., Sprando, R.L., Black, T.N., Olejnik, N., Eppley, R.M., Hines, F.A., and Ruggles, D.I. 2004 abstr. Effects of deoxynivalenol (DON, vomitoxin) on *in utero* development of rats. Abstract No. P70. Birth Defects Res, 70(5): 317.

Collins, T.F., Sprando, R.L., Black, T.N., Olejnik, N., Eppley, R.M., Hines, F.A., Rorie, J., and Ruggles, D.I. 2006. Effects of deoxynivalenol (DON, vomitoxin) on *in utero* development in rats. Food Chem Toxicol, 44(6):747-757. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=16325976. Last accessed on July 7, 2008.

Cote, L.M., Reynolds, J.D., Vesonder, R.F., Buck, W.B., Swanson, S.P., Coffey, R.T., and Brown, D.C. 1984. Survey of vomitoxin-contaminated feed grains in Midwestern United States, and associated health problems in swine. J Am Vet Med Assoc, 184(2):189-192. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/6230342?ordinalpos=1&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 9, 2008.

Czerwiecki, L., and Wilczyńska, G. 2003. Determination of deoxynivalenol in cereals by HPLC-UV. Mycotoxin Res, 19(1):31-34. Abstract from CABA 2004:48184.

Danicke, S., Valenta, H., and Doll, S. 2004. On the toxicokinetics and the metabolism of deoxynivalenol (DON) in the pig. Arch Anim Nutr, 58(2):169-180. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=15195910. Last accessed on March 5, 2009.

Debouck, C., Haubruge, E., Bollaerts, P., van Bignoot, D., Brostaux, Y., Werry, A., and Rooze, M. 2001. Skeletal deformities induced by the intraperitoneal administration of deoxynivalenol (vomitoxin) in mice. Int Orthop, 25(3):194-198. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/11482540?ordinalpos=3&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 9, 2008.

Doll, S., Danicke, S., and Schnurrbusch, U. 2003a. The effect of increasing concentrations of *Fusarium* toxins in the diets for piglets on histological parameters of the uterus. Mycotoxin Res, 19(1):73-76. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/15595624?ordinalpos=2&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 9, 2008.

Doll, S., Danicke, S., Ueberschar, K.H., Valenta, H., and Flachowsky, G. 2003b. *Fusarium* toxin residues in physiological samples of piglets. Mycotoxin Res, 19(2):171-175. Abstract from CABA 2004:128197.

Doll, S., Danicke, S., and Valenta, H. 2008. Residues of deoxynivalenol (DON) in pig tissue after feeding mash or pellet diets containing low concentrations. Mol Nutr Food Res, 52(6):727-734. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=18465777. Last accessed on March 4, 2009.

Donley, A. 2004. Vomitoxin causing headaches for U.S. flour millers. Internet Address: http://www.grainnet.com/info/articles.html?type=bn&ID=23391. Last accessed on August 25, 2004.

EC (European Community). 2003. European Community comments for the Codex Committee on Food Additives and Contaminants, 35th Session, March 17-21, 2003, Arusha, Tanzania. Agenda Item 16(j): Discussion paper on deoxynivalenol (CX/FAC 03/35). Internet address: http://europa.eu.int/comm/food/fs/ifsi/eupositions/ccfac/ccfac_ec-comments03-35_item16j_en.pdf. Last accessed on June 25, 2008.

El-Sayed, A.M.A.A, Soher, E.A., and Sahab, A.F. 2003. Occurrence of certain mycotoxins in corn and corn-based products and thermostability of *fumonisin* B1 during processing. Nahrung, 47(4):222-225. Abstract from PubMed 13678256. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/13678256?ordinalpos=2&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 3, 2008.

Eskola, M. 2002. Study on trichothecenes, zearalenone and ochratoxin A in Finnish cereals: Occurrence and analytical techniques. Academic dissertation. University of Helsinki Department of Applied Chemistry and Microbiology. Internet address: http://ethesis.helsinki.fi/julkaisut/maa/skemi/vk/eskola/studyont.pdf. Last accessed on June 27, 2008.

FDA (Food and Drug Administration). 2008. Compliance program guidance manual. Report No. 7307.001. Chapter 07 – Molecular Biology and Natural Toxins. Mycotoxins in domestic and imported foods, FY 07/08. Internet address: http://www.cfsan.fda.gov/~acrobat/cp07001.pdf. Last accessed on July 3, 2008.

Gimeno, A. Undated. Mycotoxins—Deoxynivalenol, A risk mycotoxin for children. Analytical Methods. Deoxynivalenol levels in wheat-based food products. Internet address: http://www.engormix.com/deoxynivalenol_a_risk_mycotoxin_e_articles_58_MYC.htm. Last accessed on June 27, 2008.

Gouze, M.E., Leroux, M., Dedieu, G., Oswald, I.P., and Galtier, P. 2003 abstr. Compared toxicological effects in mice receiving low levels of deoxynivalenol or nivalenol. Abstract No. G-06 (0068). J Vet Pharmacol Therap, 26(1):255-256.

Gouze, M.E., Laffitte, J., Dedieu, G., Galinier, A., Thouvenot, J.P., Oswald, I.P., and Galtier, P. 2005. Individual and combined effects of low oral doses of deoxynivalenol and nivalenol in mice. Cell Mol Biol (Noisy-le-grand), 51 Suppl:OL809-OL817. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=16375817. Last accessed on July 7, 2008.

Gouze, M.E., Laffitte, J., Rouimi, P., Loiseau, N., Oswald, I.P., and Galtier, P. 2006. Effect of various doses of deoxynivalenol on liver xenobiotic metabolizing enzymes in mice. Food Chem Toxicol, 44(4):476-483. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=16209902. Last accessed on July7, 2008.

Gouze, M.E., Laffitte, J., Pinton, P., Dedieux, G., Galinier, A., Thouvenot, J.P., Loiseau, N., Oswald, I.P., and Galtier, P. 2007. Effect of subacute oral doses of nivalenol on immune and metabolic defence systems in mice. Vet Res, 38(4):635-646. Abstract from PubMed 17565910. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=17565910. Last accessed on July 7, 2008.

Goyarts, T., and Danicke, S. 2006. Bioavailability of the *Fusarium* toxin deoxynivalenol (DON) from naturally contaminated wheat for the pig. Toxicol Lett, 163(3):171-182. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=16326049. Last accessed on March 5, 2009.

Goyarts, T., Danicke, S., Valenta, H.,a nd Ueberschar, K.H. 2007. Carry-over of *Fusarium* toxins (deoxynivalenol and zearalenone) from naturally contaminated wheat to pigs. Food Addit Contam, 24(4):369-380. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=17454110. Last accessed on March 4, 2009.

Gray, J.S., and Pestka, J.J. 2007. Transcriptional regulation of deoxynivalenol-induced IL-8 expression in human monocytes. Toxicol Sci, 99(2):502-511. Abstract from PubMed 17636245. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=17636245. Last accessed on July 7, 2008.

Hammond, B.G., Campbell, K.W., Pilcher, C.D., Degooyer, T.A., Robinson, A.E., Mcmillen, B.L., Spangler, S.M., Riordan, S.G., Rice, L.G., and Richard, J.L. 2004. Lower fumonisin mycotoxin levels in the grain of Bt corn grown in the United States in 2000-2002. J Agric Food Chem, 52(5):1390-1397. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=14995151. Last accessed on March 5, 2009.

Hanschmann, G., and Krieg, D. 2006. The fate of *Fusarium* toxins in the course of the synthesis of bioethanol from contaminated grain (German). Mycotoxin Res, 22(3):174-177. Abstract from CABA 2007:51294.

Hooker, D.C., Schaafsma, A.W., and Tamburic-Ilincic, L. 2002. Using weather variables pre- and post-handling to predict deoxynivalenol content in winter wheat. Plant Dis, 86:611-619.

Horugel, K., and Vergara, H. 2003. Influence of mycotoxins on growth and onset of puberty in growing female pigs (Ger.). Praktische Tierarzt, 84(8):611-614. Abstract from CABA 2003:164307.

Hsia, C.C., Wu, Z.Y., Li, Y.S., Zhang, P., and Sun, Z.T. 2004. Nivalenol, a main *Fusarium* toxin in dietary foods from high-risk areas of cancer of esophagus and gastric cardia in China, induced benign and malignant tumors in mice. Oncol Rep, 12(2):449-456. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/15254715?ordinalpos=1&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 9, 2008.

Huang, X.H., Zhang, X.H., Li, Y.H., Wang, J.L., Yan, X., Xing, L.X., and Wang, F.R. 2004. Carcinogenic effects of sterigmatocystin and deoxynivalenol in NIH mice (Chin.). Zhonghua Zhong Liu Za Zhi, 26(12):705-708. Abstract from PubMed 15733384. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=15733384. Last accessed on July 7, 2008.

Humpf, H.-U., and Konigs, M. 2008 abstr. Toxicity studies with deoxynivalenol using human cells in primary culture. Abstracts of Papers, 235th ACS Nationall Meeting, New Orleans, LA, USA, April 6-10, 2008, p. AGFD-144. Abstract from TOXCENTER 20008:106786.

Hymery, N., Sibiril, Y., and Parent-Massin, D. 2006. *In vitro* effects of trichothecenes on human dendritic cells. Toxicol *In Vitro*, 20(6):899-909. Abstract from PubMed 16517116. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=16517116. Last accessed on July 7, 2008.

IARC (International Agency for Research on Cancer). 1993. Some naturally occurring substances: Food items and constituents, heterocyclic aromatic amines and mycotoxins. In: IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 56, 599 pp. IARC, Lyon, France.

IARC. 2002. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. In: IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 82, 590 pp. IARC, Lyon, France.

Instanes, C., and Hetland, G. 2004. Deoxynivalenol (DON) is toxic to human colonic, lung and monocytic cell lines, but does not increase the ige response in a mouse model for allergy. Toxicology, 204(1):13-21. Abstract from PubMed 15369845. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=15369845. Last accessed on July 7, 2008.

Islam, Z., and Pestka J.J. 2003. Role of IL-1β in endotoxin potentiation of deoxynivalenol-induced corticosterone response and leukocyte apoptosis in mice. Toxicol Sci, 74(1):93-102. Internet address: http://toxsci.oxfordjournals.org/cgi/reprint/74/1/93. Last accessed on July 8, 2008.

Islam, Z., and Pestka, J.J. 2006. LPS priming potentiates and prolongs proinflammatory cytokine response to the trichothecene deoxynivalenol in the mouse. Toxicol Appl Pharmacol, 211(1):53-63. Abstract from PubMed 16009389. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=16009389. Last accessed on July 7, 2008.

Ivanova, L., Skjerve, E., Eriksen, G.S., and Uhlig, S. 2006. Cytotoxicity of enniatins A, A1, B, B1, B2 and B3 from *Fusarium avenaceum*. Toxicon, 47(8):868-876. Abstract from PubMed 16730043. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=16730043. Last accessed on July 7, 2008.

Iverson, F., Armstrong, C., Nera, E., Truelove, J., Fernie, S., Scott, P., Stapley, R., Hayward, S., and Gunner, S. 1995. Chronic feeding study of deoxynivalenol in B6C3$F_1$ male and female mice. Teratog Carcinog Mutagen, 15(6):283-306. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=8732880. Last accessed on July 11, 2008.

Jadamus, A., and Schneider, D. 2002. Long-term effect of fusariotoxins on the reproduction performance of sows (Ger.). Kraftfutter, 85(10):396-405. Abstract from CABA 2002:211987.

Jarvis, B.B., and Miller, J.D. 2005. Mycotoxins as harmful indoor air contaminants. Appl Microbiol Biotechnol, 66(4):367-372. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=15565335. Last accessed on March 5, 2009.

JECFA (Joint FAO/WHO Expert Committee on Food Additives). 2001a. Deoxynivalenol. Safety evaluation of certain mycotoxins in food. WHO Food Additives Series No. 47. World Health Organization (WHO), Geneva—International Programme on Chemical Safety (IPCS). Internet address: http://www.inchem.org/documents/jecfa/jecmono/v47je05.htm. Last accessed on March 16, 2004.

JECFA. 2001b. Fumonisins. Safety evaluation of certain mycotoxins in food. WHO Food Additives Series No. 47. World Health Organization (WHO), Geneva—International Programme on Chemical Safety (IPCS). Internet address: http://www.inchem.org/documents/jecfa/jecmono/v47je03.htm. Last accessed on September 13, 2004.

JECFA. 2001c. T-2 and HT-2 Toxins. Safety evaluation of certain mycotoxins in food. WHO Food Additives Series No. 47. World Health Organization (WHO), Geneva—International Programme on Chemical Safety (IPCS). Internet address: http://www.inchem.org/documents/jecfa/jecmono/v47je06.htm. Last accessed on September 13, 2004.

JECFA. 2001d. WHO Food Additives Series: 47; Safety evaluation of certain mycotoxins in food. World Health Organization (WHO), Geneva—International Programme on Chemical Safety (IPCS). Internet address: http://www.inchem.org/documents/jecfa/jecmono/v47je01.htm. Last accessed on June 30, 2008.

JECFA. 2002. Evaluation of certain mycotoxins in food. WHO Technical Report Series No. 906. 56$^{th}$ report of the Joint FAO/WHO Expert Committee on Food Additives. WHO, Geneva, Switzerland, 62 pp.

Jia, Q., Shi, Y., Bennink, M.B., and Pestka, J.J. 2004. Docosahexaenoic acid and eicosapentaenoic acid, but not alpha-linolenic acid, suppress deoxynivalenol-induced experimental IgA nephropathy in mice. J Nutr, 134(6):1353-1361.

Jia, Q., Zhou, H.R., Shi, Y., and Pestka, J.J. 2006. Docosahexaenoic acid consumption inhibits deoxynivalenol-induced CREB/ATF1 activation and IL-6 gene transcription in mouse macrophages. J Nutr, 136(2):366-372. Abstract from PubMed 16424113. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=16424113. Last accessed on July 7, 2008.

Juodeikiene, G., Kunigelis, V., Vidmantiene, D., and De Koe, W.J. 2004. Acoustic screening method for the determination of deoxynivalenol (DON) in wheat. Vet Zootech, 25:52-59. Abstract from CABA 2004:93026.

Keese, C., Meyer, U., Valenta, H., Schollenberger, M., Starke, A., Weber, I.-A., Rehage, J., Breves, G., and Danicke, S. 2008. No carry over of unmetabolised deoxynivalenol in milk of dairy cows fed high concentrate proportions. Mol Nutr Food Res, 52(12):1514-1529. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=18803258. Last accessed on March 5, 2009.

Kim, E.-J., Jeong, S.-H., Ku, H.-O., Kang, H.-G., and Cho, J.-H. 2007. Clinical and toxico-pathological parameters for deoxynivalenol intoxication in B6C3F$_1$ mice. J Toxicol Public Health, 23(4):353-362. Abstract from TOXCENTER 2008:74867.

King, S.J., Oh, S.S., Park, J.H., Kim, H.K., and Chung, D.H. 2001. Screening of deoxynivalenol producing strains from agricultural products by immunoanalytical method (Korean). Han'guk Hwankyong Uisaeng Hakhoechi, 27(4):35-40. Abstract from TOXCENTER 2004:16716.

Kinser, S., Li, M., Jia, Q., and Pestka, J.J. 2005. Truncated deoxynivalenol-induced splenic immediate early gene response in mice consuming (n-3) polyunsaturated fatty acids. J Nutr Biochem, 16(2):88-95. Abstract from PubMed 15681167. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=15681167. Last accessed on July 7, 2008.

Konigs, M., Lenczyk, M., Schwerdt, G., Holzinger, H., Gekle, M., and Humpf, H.U. 2007. Cytotoxicity, metabolism and cellular uptake of the mycotoxin deoxynivalenol in human proximal tubule cells and lung fibroblasts in primary culture. Toxicology, 240(1-2):48-59. Abstract from PubMed 17825972. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=17825972. Last accessed July 7, 2008.

Konigs, M., Schwerdt, G., Gekle, M., and Humpf, H.U. 2008. Effects of the mycotoxin deoxynivalenol on human primary hepatocytes. Mol Nutr Food Res, 52(7):830-839. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=18618482. Last accessed on March 5, 2009.

Kos, G., Lohninger, H., and Krska, R. 2002. Fourier transform mid-infrared spectroscopy with attenuated total reflection (FT-IR/ATR) as a tool for the detection of *Fusarium* fungi on maize. Vib Spectrosc, 29(1-2):115-119. Abstract from TOXCENTER 2002:148331.

Krska, R., Szente, E., Freudenschuss, M., Hametner, C., and Zollner, P. 2004. Purity assessment of commercially available crystalline deoxynivalenol. J AOAC Int, 87(4):909-919. Abstract from PubMed 15295885. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/15295885?ordinalpos=7&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on June 26, 2008.

Krysinska-Traczyk, E., Perkowski, J., Kostecki, M., Dutkiewicz, J., and Kiecana, I. 2003. Filamentous fungi and mycotoxins as potential occupational risk factors among farmers harvesting various crops (Polish). Med Pr, 54(2):133-138. Abstract from PubMed 12923995. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/12923995?ordinalpos=8&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 3, 2008.

Kubosaki, A., Aihara, M., Park, B.J., Sugiura, Y., Shibutani, M., Hirose, M., Suzuki, Y., Takatori, K., and Sugita-Konishi, Y. 2008. Immunotoxicity of nivalenol after subchronic dietary exposure to rats. Food Chem Toxicol, 46(1):253-258. Abstract from PubMed 17881110. PubMed abstract Internet address:

http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=17881110. Last accessed on July 7, 2008.

Lambert, L.A., Hines, F.A., and Eppley, R.M. 1995. Lack of initiation and promotion potential of deoxynivalenol for skin tumorigenesis in Sencar mice. Food Chem Toxicol, 33(3):217-222. Abstract from PubMed 7896232. Pubmed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/7896232?ordinalpos=9&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 11, 2008.

Lancova, K., Hajslova, J., Poustka, J., Krplova, A., Zachariasova, M., Dostalek, P., and Sachambula, L. 2008. Transfer of *Fusarium* mycotoxins and 'masked' deoxynivalenol (deoxynivalenol-3-glucoside) from field barley through malt to beer. Food Addit Contam Part A Chem Anal Control Expo Risk Assess, 25(6):732-744. Abstract from PubMed 18484301. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=18484301. Last accessed on March 5, 2009.

Landgren, C.A., Hendrich, S., and Kohut, M.L. 2006. Low-level dietary deoxynivalenol and acute exercise stress result in immunotoxicity in BALB/c mice. J Immunotoxicol, 3(4):173-178.

Larsen, J.C., Hunt, J., Perrin, I., and Ruckenbauer, P. 2004. Workshop on trichothecenes with a focus on DON: summary report. Toxicol Lett, 153(1):1-22. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=15342076. Last accessed on February 22, 2009.

Lawlor, P.G., and Lynch, P.B. 2001. Mycotoxins in pig feeds. 2: Clinical aspects. Irish Vet J, 54(4):172-176.

Leblanc, J.C., Tard, A., Volatier, J.L., and Verger, P. 2005. Estimated dietary exposure to principal food mycotoxins from the first French total diet study. Food Addit Contam, 22(7):652-672. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=16019841. Last accessed in March 5, 2009.

Li, B., and Guo, H. 2000. Study on the combined toxicity of aflatoxin B1 and deoxynivalenol (Chin.). Wei Sheng Yan Jiu (J Hyg Res), 29(6):393-395. Abstract from PubMed 12520966. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/12520966?ordinalpos=1&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 3, 2008.

Li, Y., Zhang, X., Wang, J., Yan, X., Huang, X., Yang, J., Liu, Y., and Wang, F. 2002. Effects of deoxynivalenol on apoptosis and proliferation of mouse thymocytes *in vivo* (Chin.). Zhongguo Bingli Shengli Zazhi, 18(7):778-781. Abstract from TOXCENTER 2004:1177.

Li, M., Cuff, C.F., and Pestka, J. 2005. Modulation of murine host response to enteric reovirus infection by the trichothecene deoxynivalenol. Toxicol Sci, 87(1):134-145. Abstract from PubMed 15958657. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=15958657. Last accessed on July 7, 2008.

Li, M., Harkema, J.R., Cuff, C.F., and Pestka, J.J. 2007. Deoxynivalenol exacerbates viral bronchopneumonia induced by respiratory reovirus infection. Toxicol Sci, 95(2):412-426. Internet address: http://toxsci.oxfordjournals.org/cgi/reprint/95/2/412. Last accessed on March 5, 2009.

Lin, L., and Sung, S.Q. 2004. Study on DNA damage and repair induced by deoxynivalenol in cultured Vero cell with Comet assay. Chin J Control Endemic Dis, 19(3):139-141. Cited by Ma and Guo (2008).

Liu, J., Xing, X., Xing, L.-X., Zhou, B.-J., Yan, X., Wng, J.-L., Li, Y.-H., and Zhang, X.-H. 2007. Effects of deoxynivalenol on cell cycle and apoptosis of human gastric carcinoma cell line HGC-27 *in vitro* (Chinese). Zhongliu Fangzhi Yanjiu, 34(12):897-900. Abstract from TOXCENTER 2008:271909.

Lombaert, G.A., Pellaers, P., Roscoe, V., Mankotia, M, Neil, R., and Scott, P.M. 2003. Mycotoxins in infant cereal foods from the Canadian retail market. Food Addit Contam, 20(5):494-504. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/12775469?ordinalpos=4&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 9, 2008.

Ma, Y.Y., and Guo, H.W. 2008. Mini-review of studies on the carcinogenicity of deoxynivalenol. Environ Toxicol Pharmacol, 25(1):1-9.

MacDonald, S., Prickett, T.J., Wildey, K.B., and Chan, D. 2004. Survey of ochratoxin A and deoxynivalenol in stored grains from the 1999 harvest in the UK. Food Addit Contam, 21(2):172-181. Abstract from PubMed 14754640. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/14754640?ordinalpos=2&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on June 26, 2008.

Madhyastha, M.S., Marquardt, R.R., and Abramson, D. 1994. Structure-activity relationships and interactions among trichothecene mycotoxins as assessed by yeast bioassay. Toxicon, 32(9):1147-1152. Abstract from PubMed 7801350. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/7801350?ordinalpos=2&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on June 26, 2008.

Manthey, F.A., Wolf-Hall, C.E., Yalla, S., Vijayakumar, C., and Carlson, D. 2004. Microbial loads, mycotoxins , and quality of durum wheat from the 2001 harvest of the northern plains region of the United States. J Food Prot, 67(4):772-780. Abstract from PubMed 15083730. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/15083730?ordinalpos=12&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on June 26, 2008.

Maragos, C.M, Jolley, M.E., and Nasir, M.S. 2002. Fluorescence polarization as a tool for the determination of deoxynivalenol in wheat. Food Addit Contam, 19(4):400-407. Abstract from PubMed 11962698. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/11962698?ordinalpos=8&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 3, 2008.

Maresca, M., Yahi, N., Younes-Sakr, L., Boyron, M., Caporiccio, B., and Fantini, J. 2008. Both direct and indirect effects account for the pro-inflammatory activity of enteropathogenic mycotoxins on the human intestinal epithelium: Stimulation of interleukin-8 secretion, potentiation of interleukin-β effect and increase in the transepithelial passage of commensal bacteria. Toxicol Appl Pharmacol, 228(1):84-92. Abstract from PubMed 18308354. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=18308354. Last accessed on July 7, 2008.

Mayer, S., Curtui, V., Usleber, E., and Gareis, M. 2007a. Airborne mycotoxins in dust from grain elevators. Mycotoxin Res, 23(2):94-100. Abstract from CABA 2007:205489.

Mayer, S., Gareis, M., Degen, G.H., Blaszkewicz, M., Curtui, V., and Usleber, E. 2007b. Exposure to airborne mycotoxins at grain/corn storage facilities and mycotoxin concentrations in the blood of grain storage workers (German). Gefahrst Reinhalt L, 67(4):119-125. Abstract from CABA 2007:106625.

McMullen, M.P., and Stack, R.W. 1999. *Fusarium* head blight (scab) of small grains. PP-804 (Revised). Internet address: http://www.ext.nodak.edu/extpubs/plantsci/smgrains/pp804w.htm. Last updated in December 1999. Last accessed on March 22, 2004.

McMullen, M., Halley, S., Schatz, B., Meyer, S., Jordahl, J., and Ransom, J. 2008. Integrated strategies for *Fusarium* head blight management in the United States. Cereal Res Commun, 36:563-568. Abstract from BIOSIS 2008:624155.

Meky, F.A., Hardie, L.J., Evans, S.W., and Wild, C.P. 2001. Deoxynivalenol-induced immunomodulation of human lymphocyte proliferation and cytokine production. Food Chem Toxicol, 39(8):827-836. Abstract from PubMed 11434990. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/11434990?ordinalpos=12&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 3, 2008.

Meky, F.A., Turner, P.C., Ashcroft, A.E., Miller, J.D., Qiao, Y.-L., Roth, M.J., and Wild, C.P. 2003. Development of a urinary biomarker of human exposure to deoxynivalenol. Food Chem Toxicol, 41(2):265-273. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/12480302?ordinalpos=3&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 9, 2008.

Minervini, F., Fornelli, F., and Flynn, K.M. 2004. Toxicity and apoptosis induced by the mycotoxins nivalenol, deoxynivalenol and fumonisin B1 in a human erythroleukemia cell line. Toxicol *In Vitro*, 18(1):21-28. Abstract from PubMed 14630058. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=14630058. Last accessed on July 7, 2008.

Moon, Y., and Pestka J.J. 2002. Vomitoxin-induced cyclooxygenase-2 gene expression in macrophages mediated by activation of ERK and p38 but not JNK mitogen-activated protein kinases. Toxicol Sci, 69(2):373-382. Internet address: http://toxsci.oxfordjournals.org/cgi/reprint/69/2/373. Last accessed on July 8, 2008.

Moon, Y., and Pestka, J.J. 2003a. Cyclooxygenase-2 mediates interleukin-6 upregulation by vomitoxin (deoxynivalenol) *in vitro* and *in vivo*. Toxicol Appl Pharmacol, 187(2):80-88. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/12649040?ordinalpos=5&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 9, 2008.

Moon, Y., and Pestka, J.J. 2003b. Deoxynivalenol-induced mitogen-activated protein kinase phosphorylation and IL-6 expression in mice suppressed by fish oil. J Nutr Biochem, 14(12):717-726. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/14690764?ordinalpos=2&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 9, 2008.

Moon, Y., Yang, H., and Lee, S.H. 2007. Modulation of early growth response gene 1 and interleukin-8 expression by ribotoxin deoxynivalenol (vomitoxin) via ERK1/2 in human epithelial intestine 407 cells. Biochem Biophys Res Commun, 362(2):256-262. Abstract fromPubMed 17707346. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=17707346. Last accessed on July 8, 2008.

Nagy, M.A., and Kuldau, G.A. 2003. Mycotoxicogenic fungi and mycotoxins in Pennsylvania corn silage. Phytopathology, 93(6):S97-S98. Abstract from BIOSIS 2003:393026.

Nasri, T., Bosch, R.R., Voorde, S., and Fink-Gremmels, J. 2006. Differential induction of apoptosis by type A and B trichothecenes in Jurkat T-lymphocytes. Toxicol *In Vitro*, 20(6):832-840. Abstract from PubMed 16472964. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=16472964. Last accessed on July 7, 2008.

Nedelnik, J. 2002. Damage to corn by fungi of the genus *Fusarium* and the presence of fusariotoxins. Plant Protect Sci, 38(2):46-54. Abstract from CABA 2002:170284.

Ngundi, M.M., Qadri, S.A., Wallace, E.V., Moore, M.H., Lassman, M.E., Shriver-Lake, L.C., Ligler, F.S., and Taitt, C.R. 2006. Detection of deoxynivalenol in foods and indoor air uring an array biosensor. Environ Sci Technol, 40(7):2352-2356. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=16646473. Last accessed on March 6, 2009.

Nordby, K.C., Halstensen, A.S., Elen, O., Clasen, P.E., Langseth, W., Kristensen, P., and Eduard, W. 2004. Trichothecene mycotoxins and their determinants in settled dust related to grain production. Ann Agric Environ Med, 11(1):75-83. Internet address: http://www.aaem.pl/pdf/11075.pdf. Last accessed on July 8, 2008.

North Dakota State University. 2000. Feeding vomitoxin-infested grain may be best solution, NDSU specialist says. Internet address: http://www.scabusa.org/pdfs/000914_PressRelease.PDF. Last accessed on June 26, 2008.

NTP (National Toxicology Program). 1982. Carcinogenesis bioassay of zearalenone (CAS No. 17924-92-4) in F344/N rats and B6C3F$_1$ mice (feed study). TR-235. Internet address: http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr235.pdf. Last accessed on July 3, 2008.

NTP. 2001. Toxicology and carcinogenesis studies of fumonisin B$_1$ (CAS No. 116355-83-0) in F344/N rats and B6C3F$_1$ mice (feed studies). TR-496 Internet address: http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr496.pdf. Last accessed on July 3, 2008.

Papadopoulou-Bouraoui, A., Vrabcheva, T., Valzacchi, S., Stroka, J. and Anklam, E. 2004. Screening survey of deoxynivalenol in beer from European market by an enzyme-linked immunosorbent assay. Food Addit Contam, 21(6):607-617. Abstract from PubMed 15204540. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/15204540?ordinalpos=3&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on June 26, 2008.

Penner, K.M., Gray, J.S., and Pestka, J.J. 2003 abstr. Human cytokine mRNA response to deoxynivalenol (vomitoxin) using whole blood cultures. Abstract No. 1231 (SOT 2003 Annual Meeting). Toxicol Sci, 72(Suppl. 1):253.

Perkowski, J. 2000. Mycotoxins in raw brewery materials and beer and during brewing process (Polish). Przemysl Fermentacyjny i Owocowo Warzywny, 44(11):14-16. Abstract from TOXCENTER 2001:75809.

Pestka, J.J. 2003. Deoxynivalenol-induced IgA production and IgA nephropathy-aberrant mucosal immune response with systemic repercussions. Toxicol Lett, 140-141:287-295. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/12676476?ordinalpos=2&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 9, 2008.

Pestka, J.J., and Dong, W. 1994. Progressive serum IgE elevation in the B6C3F$_1$ mouse following withdrawal of dietary vomitoxin (deoxynivalenol). Fundam Appl Toxicol, 22(2):314-316. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/8005381?ordinalpos=6&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 9, 2008.

Pestka, J.J., and Zhou, H.R. 2000. Interleukin-6-deficient mice refractory to IgA dysregulation but not anorexia induction by vomitoxin (deoxynivalenol) ingestion. Food Chem Toxicol, 38(7):565-575. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/10942317?ordinalpos=3&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 9, 2008.

Pestka, J.J., and Zhou, H.R. 2002. Effects of tumor necrosis factor type 1 and 2 receptor deficiencies on anorexia, growth and IgA dysregulation in mice exposed to the trichothecene vomitoxin. Food Chem Toxicol, 40(11):1623-1631. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/12176089?ordinalpos=1&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 9, 2008.

Pestka, J., and Zhou, H.R. 2006. Toll-like receptor priming sensitizes macrophages to proinflammatory cytokine gene induction by deoxynivalenol and other toxicants. Toxicol Sci, 92(2):445-455. Abstract from PubMed 16687389. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=16687389. Last accessed on July 7, 2008.

Pestka, J.J., Moorman, M.A., and Warner, R.L. 1990. Altered serum immunoglobulin response to model intestinal antigens during dietary exposure to vomitoxin (deoxynivalenol). Toxicol Lett, 50(1):75-84. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/2296780?ordinalpos=2&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 9, 2008.

Pestka, J.J., Zhou, H.-R., Jia, Q., and Timmer, A.M. 2002. Dietary fish oil suppresses experimental immunoglobulin A nephropathy in mice. J Nutr, 132(2):261-269. Internet address: http://jn.nutrition.org/cgi/reprint/132/2/261. Last accessed on July 8, 2008.

Pestka, J.J., Uzarski, R.L., and Islam, Z. 2005. Induction of apoptosis and cytokine production in the Jurkat human T cells by deoxynivalenol: role of mitogen-activated protein kinases and comparison to other 8-ketotrichothecenes. Toxicology, 206(2):207-219. Abstract from PubMed 15588914. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=15588914. Last accessed on July 7, 2008.

Pestka, J.J., Islam, Z., and Amuzie, C.J. 2008. Immunochemical assessment of deoxynivalenol tissue distribution following oral exposure in the mouse. Toxicol Lett, 178(2):83-87. Abstract from EMBASE 2008205778.

Pieters, M.N., Freijer, J., Baars, A.J., and Slob, W. 2001. Risk assessment of deoxynivalenol in food: An assessment of exposure and effects in the Netherlands. RIVM Report 388802 022. National Institute of Public Health and the Environment. Internet address: http://www.rivm.nl/bibliotheek/rapporten/388802022.pdf. Last accessed on June 26, 2008.

Poapolathep, A., Sugita-Konishi, Y., Phitsanu, T., Doi, K., and Kumagai, S. 2004. Placental and milk transmission of trichothecene mycotoxins, nivalenol and fusarenon-X, in mice. Toxicon, 44(1):111-113. Abstract from PubMed 15225570. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/15225570?ordinalpos=3&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 16, 2008.

Prelusky, D.B. 1997. Effect of intraperitoneal infusion of deoxynivalenol on feed consumption and weight gain in the pig. Nat Toxins, 5:121-125. Cited by Szkudelska et al. (2002).

Pronk, M.E.J., Schothorst, R.C., and van Egmond, H.P. 2002. Toxicology and occurrence of nivalenol, fusarenon X, diacetoxyscirpenol, neosolaniol, and 3- and 15-acetyldeoxynivalenol: A review of six trichothecenes. RIVM Report No. 388802024/2002. Internet address: http://www.rivm.nl/bibliotheek/rapporten/388802024.pdf. Last accessed on June 26, 2008.

Ranzenigo, G., Caloni, F., Cremonesi, F., and Spicer, L.J. 2006 abstr. *Fusarium* mycotoxins affect progesterone and estradiol production of pig granulosa cells *in vitro*. Abstract No. P226. Reprod Domest Anim, 41(4):365.

Razzazi-Fazeli, E., Bohm, J., Jarukamjorn, K., Zentek, J., and Shirai, Y. 2003. Simultaneous determination of major B-trichothecenes and the de-epoxy-metabolite of deoxynivalenol in pig urine and maize using high-performance liquid chromatography-mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci, 796(1):21-33. Abstract from PubMed 14552813. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/14552813?ordinalpos=13&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on June 26, 2008.

Registry. 2004. RN 514-10-8. Record entered STN on November 16, 1984. Database available from the American Chemical Society on STN International.

Robl, M.G., Garthoff, L.H., and Hinton, D.M. 2005 abstr. Acute histopathology and total blood parameters in young male rats given bacterial (lipopolysaccharide-LPS) or fungal (deoxynivalenol-DON) toxins. Board No. J-35. 11th Annual FDA Science Forum, April 27-28, 2005. Internet address: http://www.accessdata.fda.gov/scripts/oc/scienceforum/sf2005/Search/preview.cfm?abstract_id=330&backto=author. Last accessed on July 7, 2008.

Ryu, D., and Bullerman, L.B. 2008 abstr. Mycotoxins of concern in imported grains. Abstracts of Papers, 235th ACS National Meeting, New Orleans, LA, United States, April 6-10, 2008.

Sahu, S.C., Garthoff, L.H., Robl, M.G., Chirtel, S.J., Ruggles, D.I., Flynn, T.J., and Sobotka, T.J. 2008. Rat liver clone-9 cells in culture as a model for screening hepatotoxic potential of food-related products: Hepatotoxicity of deoxynivalenol. J Appl Toxicol [Epublication ahead of print]. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=18300328. Last accessed on July 7, 2008.

Sakai, A., Suzuki, C., Masui, Y., Kuramashi, A., Takatori, K., and Tanaka, N. 2007. The activities of mycotoxins derived from *Fusarium* and related substances in a short-term transformation assay using v-Ha-ras-transfected BALB/3T3 cells (Bhas 42 cells). Mutat Res, 630(1-2):103-111. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=17499015. Last accessed on July 7, 2008.

Schaafsma, A.W., Tamburic-Ilinic, L., Miller, J.D., and Hooker, D.C. 2001. Agronomic considerations for reducing deoxynivalenol in wheat grain. Can J Plant Pathol, 23(3):279-285. Abstract from AGRICOLA 2002:27510.

Schollenberger, M., Jara, H.T., Suchy, S., Drochner, W., and Muller, H.M. 2002. *Fusarium* toxins in wheat flour collected in an area in southwest Germany. Int J Food Microbiol, 72(1-2):85-89. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/11843417?ordinalpos=4&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 9, 2008.

Schothorst, R.C., and Jekel, A.A. 2003. Determination of trichothecenes in beer by capillary gas chromatography with flame ionization detection. Food Chem, 82(3):475-479. Abstract from CABA 2003:152720.

Schothorst, R.C., Jekel, A.A., Van Egmond, H.P., De Mul, A., Boon, P.E., and Van Klaveren, J.D. 2005. Determination of trichothecenes in duplicate diets of young children by capillary gas chromatography with mass spectrometric detection. Food Addit Contam, 22(1):48-55. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=15895611. Last accessed on March 5, 2009.

Scott, P.M. 1996. Mycotoxins transmitted into beer from contaminated grains during brewing. J AOAC Int, 79(4):875-882. PubMed abstract Internet address:

http://www.ncbi.nlm.nih.gov/pubmed/8757446?ordinalpos=1&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 9, 2008.

Scott, P.M., Kanhere, S.R., Dexter, J.E., Brennan, P.W., and Trenholm, H.L. 1984. Distribution of the trichothecene mycotoxin deoxynivalenol (vomitoxin) during the milling of naturally contaminated hard red spring wheat and its fate in baked products. Food Addit Contam, 1(4):313-323. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/6537355?ordinalpos=14&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 9, 2008.

Sergent, T., Parys, M., Garsou, S., Pussemier, L., Schneider, Y.J., and Larondelle, Y. 2006. Deoxynivalenol transport across human intestinal Caco-2 cells and its effects on cellular metabolism at realistic intestinal concentrations. Toxicol Lett, 164(2):167-176. Abstract from PubMed 16442754. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=16442754. Last accessed on July 7, 2008.

Shi, Y., and Pestka, J.J. 2006. Attenuation of mycotoxin-induced IgA nephropathy by eicosapentaenoic acid in the mouse: dose response and relation to IL-6 expression. J Nutr Biochem, 17(10):697-706. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=16524712. Last accessed on July 7, 2008.

Smerak, P., Barta, I., Polivkova, Z., Bartova, J., and Sedmikova, M. 2001. Mutagenic effects of selected trichothecene mycotoxins and their combinations with aflatoxin B1. Czech J Food Sci, 19(3):90-96. Abstract from CABA 2001:107438.

Sprando, R.L., Collins, T.F., Black, T.N., Olejnik, N., Rorie, J.I., Eppley, R.M., and Ruggles, D.I. 2005. Characterization of the effect of deoxynivalenol on selected male reproductive endpoints. Food Chem Toxicol, 43(4):623-635. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=15721211. Last accessed on July 7, 2008.

Starkey, D.E., Ward, T.J., Aoki, T., Gale, L.R., Kistler, H.C., Geiser, D.M., Suga, H., Toth, B., Varga, J., and O'Donnell, K. 2007. Global molecular surveillance reveals novel *Fusarium* head blight species and trichothecene toxin diversity. Fungal Genet Biol, 44(11):1191-1204. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=17451976. Last accessed on March 5, 2009.

Sudakin, D., and Fallah, P. 2008. Toxigenic fungi and mycotoxins in outdoor, recreational environments. Clin Toxicol (Phila.), 46(8):738-44. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=18615277. Last accessed on March 5, 2009.

Sugita-Konishi, Y., and Pestka, J.J. 2001. Differential upregulation of TNF-α , IL-6, and IL-8 production by deoxynivalenol (vomitoxin) and other 8-ketotrichothecenes in a human macrophage model. J Toxicol Environ Health A, 64(8):619-636. Abstract from PubMed 11766169. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/11766169?ordinalpos=1&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 3, 2008.

Sundstol Eriksen, G., Pettersson, H., and Lundh, T. 2004. Comparative cytotoxicity of deoxynivalenol, nivalenol, their acetylated derivatives and de-epoxy metabolites. Food Chem Toxicol, 42(4):619-624. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/15019186?ordinalpos=10&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 9, 2008.

Sypecka, Z., Kelly, M., and Brereton, P. 2004. Deoxynivalenol and zearalenone residues in eggs of laying hens fed with a naturally contaminated diet: effects on egg production and estimation of transmission rates from feed to eggs. J Agric Food Chem, 52:5463-5471. Abstract from PubMed 15315386. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/15315386?ordinalpos=2&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on June 26, 2008.

Szkudelska, K., Szkudelski, T., and Nogowski, L. 2002. Short-time deoxynivalenol treatment induces metabolic disturbances in the rat. Toxicol Lett, 136(1):25-31. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/12368054?ordinalpos=6&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 9, 2008.

Tajima, O., Schoen, E.D., Feron, V.J., and Groten, J.P. 2002. Statistically designed experiments in a tiered approach to screen mixtures of *Fusarium* mycotoxins for possible interactions. Food Chem Toxicol, 40(5):685-695. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/11955675?ordinalpos=1&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 9, 2008.

Takahashi, M., Shibutani, M., Sugita-Konishi, Y., Aihara, M., Inoue, K., Woo, G.H., Fujimoto, H., and Hirose, M. 2008. A 90-day subchronic toxicity study of nivalenol, a trichothecene mycotoxin, in F344 rats. Food Chem Toxicol, 46(1):125-135. Abstract from PubMed 17765382. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=17765382. Last accessed on July 7, 2008.

Turner, P.C., Rothwell, J.A., White, K.L., Gong, Y., Cade, J.E., and Wild, C.P. 2008. Urinary deoxynivalenol is correlated with cereal intake in individuals from the United Kingdom. Environ Health Perspect, 116(1):21-25. Abstract from PubMed 18197294. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=18197294. Last accessed on July 7, 2008.

USDA ARS (U.S. Department of Agriculture, Agricultural Research Service). 2005. National Programs; Food Safety (animal and plant products). FY 2004-2005. Internet address: http://www.ars.usda.gov/research/programs/programs.htm?np_code=108&docid=7589. Link available to "National Program 108. Food Safety Report 2000-2004" PDF, 31 pp. Internet address: http://www.ars.usda.gov/SP2UserFiles/Program/108/NP108AccomplishmentReport.pdf. Last accessed on July 7, 2008.

USDA FSIS (U.S. Department of Agriculture, Food Safety and Inspection Service). 2004. Codex Alimentarius Commission: 36th Session of the Codex Committee on Food Additives and Contaminants. Docket No. 03-047N. Fed Reg, 69(22):5120-5122. Internet address: http://edocket.access.gpo.gov/2004/04-2135.htm. Last accessed on July 10, 2008.

USDA GIPSA (U.S. Department of Agriculture, Grain Inspection, Packers and Stockyards Administration). Undated. Testing Trucklots of Barley and Wheat for Deoxynivalenol (DON). Internet address: http://archive.gipsa.usda.gov/programsfgis/inspwgh/don.pdf. Last accessed on July 7, 2008.

USDA GIPSA. 2002. GIPSA Backgrounder: Deoxynivalenol (DON). Internet address: http://archive.gipsa.usda.gov/newsroom/backgrounders/b-vomitox.PDF. Last accessed on July 7, 2008.

USWBSI (U.S. Wheat and Barley Scab Initiative). 2004. *Fusarium*, DON cripple wheat industry in Southwest U.S. Internet Address: http://www.scabusa.org/pdfs/forum_03_release.pdf. Last updated on January 23, 2004. Last accessed on July 7, 2008.

Uzarski, R.L., and Pestka, J.J. 2003. Comparative susceptibility of B cells with different lineages to cytotoxicity and apoptosis induction by translational inhibitors. J Toxicol Environ Health A, 66(22):2105-

2118. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/14710595?ordinalpos=1&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 9, 2008.

Uzarski, R.L., Islam, Z., and Pestka, J.J. 2003. Potentiation of trichothecene-induced leukocyte cytotoxicity and apoptosis by TNF-αand Fas activation. Chem Biol Interact, 146(2):105-119. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/14597125?ordinalpos=1&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 9, 2008.

Van De Walle, J., Romier, B., Larondelle, Y., and Schneider, Y.J. 2008. Influence of deoxynivalenol on NF-kappaB activation and IL-8 secretion in human intestinal Caco-2 cells. Toxicol Lett, 177(3):205-214. Abstract from PubMed 18343055. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=18343055. Last accessed on July 7, 2008.

Verstraete, F. 2008. Chapter 8. European Union legislation on mycotoxins in food and feed: overview of the deicison-making process and recent and future developments. In: Leslie, J.F., Bandyopadhyay, R., and Visconti, A., Eds. Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade. CAB International, Oxfordshire, UK, 512 pp. Internet address: http://books.google.com/books?id=15uD7bddmCAC&pg=PA77&lpg=PA77&dq=%22European+Union+legislation+on+mycotoxins+in+food+and+feed:+overview+of+the+decision-making+process+and+recent+and+future+developments+%22&source=bl&ots=1j_beZgKN-&sig=3-4FQI-5RJ99a9Xix5dG. Last accessed on March 5, 2009.

Waldemarson, A.H., Hedenqvist, P., Salomonsson, A.C., and Haggblom, P. 2005. Mycotoxins in laboratory rodent feed. Lab Anim, 39(2):230-235. Abstract from PubMed 15901367. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=15901367. Last accessed on July 7, 2008.

Walker, F., and Meier, B. 1998. Determination of the *Fusarium* mycotoxins nivalenol, deoxynivalenol, 3-acetyldeoxynivalenol, and 15-*O*-acetyl-4-deoxynivalenol in contaminated whole wheat flour by liquid chromatography with diode array detector and gas chromatography with electron capture detection. J AOAC Int, 81(4):741-748. Abstract from PubMed 9680699. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/9680699?ordinalpos=12&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on June 26, 2008.

Walker, S.L., Leath, S., Hagler, W.M., Jr., and Murphy, J.P. 2001. Variation among isolates of *Fusarium graminearum* associated with *Fusarium* head blight in North Carolina. Plant Dis, 85(4):404-410.

Wang, H., Zhang, X., Yang, Y., and Yan, X. 2000. Effects of deoxynivalenol on proliferation and tumor necrosis factor-alpha secretion of human peripheral blood mononuclear cells *in vitro* (Chin.) Wei Sheng Yan Jiu (J Hyg Res), 29(6):387-389. Abstract from PubMed 12520964. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/12520964?ordinalpos=1&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 3, 2008.

Ward, T.J., Clear, R.M., Rooney, A.P., O'Donnell, K., Gaba, D., Patrick, S., Starkey, D.E., Gilbert, J., Geiser, D.M., and Nowicki, T.W. 2008. An adaptive evolutionary shift in *Fusarium* head blight pathogen populations is driving the rapid spread of more toxigenic *Fusarium graminearum* in North America. Fungal Genet Biol, 45(4):473-484. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=18035565. Last accessed on March 5, 2009.

Whitaker, T.B., Richard, J.L., Giesbrecht, F.G., Slate, A.B. and Ruiz, N. 2003. Estimating deoxynivalenol in shelled corn barge lots by measuring deoxynivalenol in corn screenings. J AOAC Int, 86(6):1187-1192. Abstract from PubMed 14979701. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/14979701?ordinalpos=6&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on June 26, 2008.

Whitlow, L.W., and Hagler, W.M., Jr. 2002. Mycotoxins in feeds. Feedstuffs, 74(28):pages not provided. Internet address: http://www.oznet.ksu.edu/drought/drought/Mycotoxins%20in%20Feeds.pdf. Last accessed on June 26, 2008.

WHO (World Health Organization). 2000. Zearalenone. Safety Evaluation of Certain Food Additives and Contaminants. Food Additives Series No. 44. Internet address: http://www.inchem.org/documents/jecfa/jecmono/v44jec14.htm. Last accessed on September 14, 2004.

Wong, S., Schwartz, R.C., and Pestka, J.J. 2001. Superinduction of TNF-α and IL-6 in macrophages by vomitoxin (deoxynivalenol) modulated mRNA stabilization. Toxicology, 161(1-2):139-149. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/11295263?ordinalpos=3&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 10, 2008.

Wong, S.S., Zhou, H.R., and Pestka, J. J. 2002. Effects of vomitoxin (deoxynivalenol) on the binding of transcription factors AP-1, NF-kappaB, and NF-IL6 in raw 264.7 macrophage cells. J Toxicol Environ Health A, 65(16):1161-1180. Abstract from PubMed 12167214. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/12167214?ordinalpos=5&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 3, 2008.

Xing, X., Liu, J., Xing, L.-X., Liu, H.-M., Zhou, B.-J., Wang, J.-L., Xia, Y., and Zhang, X.-H. 2007. Effects of deoxynivalenol on proliferation and cell cycle distribution of human gastric carcinoma cell line. Xibao Shengwuxue Zazhi, 29(5):729-735. Abstract from TOXCENTER 2007:310686.

Yang, G.H., and Pestka, J.J. 2002. Vomitoxin (deoxynivalenol)-mediated inhibition of nuclear protein binding to NRE-A, an IL-2 promoter negative regulatory element, in EL-4 cells. Toxicology, 172(3):169-179. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/11893416?ordinalpos=3&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 10, 2008.

Yang, G.H., Li, S., and Pestka, J.J. 2000a. Down-regulation of the endoplasmic reticulum chaperone GRP78/BiP by vomitoxin (deoxynivalenol). Toxicol Appl Pharmacol, 162(3):207-217. PubMed abstract Internet addres: http://www.ncbi.nlm.nih.gov/pubmed/10652249?ordinalpos=31&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 10, 2008.

Yang, G.H., Jarvis, B.B., Chung, Y.J., and Pestka, J.J. 2000b. Apoptosis induction by the satratoxins and other trichothecene mycotoxins: relationship to ERK, p38 MAPK, and SAPK/JNK activation. Toxicol Appl Pharmacol, 164(2):149-160. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/10764628?ordinalpos=20&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on July 10, 2008.

Yang, H., Chung, D.H., Kim, Y.B., Choi, Y.H., and Moon, Y. 2008. Ribotoxic mycotoxin deoxynivalenol induces G2/M cell cycle arrest via p21Cip/WAF1 mRNA stabilization in human epithelial cells. Toxicology, 243(1-2):145-154. Abstract from PubMed 18006205. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=18006205. Last accessed on July 7, 2008.

Yordanova, P., Islam, Z., and Pestka, J.J. 2003 abstr. Kinetics of deoxynivalenol (vomitoxin) distribution and clearance following oral exposure in the mouse. Abstract No. 1230 (SOT 2003 Annual Meeting). Toxicol Sci, 72(Suppl. 1):253.

Yue, Y., Cao, J., Li, S., Xie, L., Sun, J., Zhang, Z., Zhang, F., and Shi, Z. 2005. The effect on proteoglycan metabolism of deoxynivalenol and selenium in the cultured human fetal chondrocytes *in vitro*. Acad J Xi'an Jiaotong Univ, 17(2):151-154. Abstract from EMBASE 2005551994.

Zhou, H., and Pestka, J.J. 2003 abstr. Deoxynivalenol-induced apoptosis mediated by p38 MAPK-dependent p53 gene induction in RAW 264.7 macrophages. Abstract No. 1601 (SOT 2003 Annual Meeting). Toxicol Sci, 72(Suppl. 1):330.

Zhou, H.R., Yan, D., Harkema, J.R., and Pestka, J.J. 1999. Amplified proinflammatory cytokine expression and toxicity in mice coexposed to lipopolysaccharide and the trichothecene vomitoxin (deoxynivalenol). J Toxicol Environ Health A, 57(2):115-136. Abstract from PubMed 10344227. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/pubmed/10344227?ordinalpos=1&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum. Last accessed on June 26, 2008.

Zhou, H.R., Islam, Z., and Pestka, J.J. 2003. Rapid, sequential activation of mitogen-activated protein kinases and transcription factors precedes proinflammatory cytokine mRNA expression in spleens of mice exposed to the trichothecene vomitoxin. Toxicol Sci, 72(1):130-142. Internet address: http://toxsci.oxfordjournals.org/cgi/reprint/72/1/130. Last accessed on July 8, 2008.

Zhou, H.R., Islam, Z., and Pestka, J.J. 2005. Induction of competing apoptotic and survival signaling pathways in the macrophage by the ribotoxic trichothecene deoxynivalenol. Toxicol Sci, 87(1):113-122. Abstract from PubMed 15976193. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=15976193. Last accessed on July 7, 2008.

Zhou, B.J., Li, Y.H., Zhang, X.H., Xing, L.X., Yan, X., Wang, J.L., Liu, J., and Xing, X. 2006a. Effects of vitamin C on apoptosis and proliferation inhibition of human peripheral blood mononuclear cells induced by deoxynivalenol *in vitro* (Chin.). Zhonghua Yu Fang Yi Xue Za Zhi, 40(5):309-313. Abstract from PubMed 17166419. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=17166419. Last accessed on July 7, 2008.

Zhou, B.J., Li, Y.H., Zhang, X.H., Xing, L.X., Yan, X., Wang, J.L., Liu, J., and Xing, X. 2006b. Effects of vitamin C on the inhibition of human leucocyte antigen class I (HLA-I) expression of human peripheral blood mononuclear cells induced by deoxynivalenol *in vitro* (Chin.). Zhonghua Yu Fang Yi Xue Za Zhi, 40(4):314-318. Abstract from PubMed 17166420. PubMed abstract Internet address: http://www.ncbi.nlm.nih.gov/sites/entrez?orig_db=PubMed&db=pubmed&cmd=Search&TransSchema=title&term=17166420. Last accessed on July 7, 2008.

Zielonka, L., Gajecki, M., Obremsni, K., and Zwierzchowski, W. 2004. Influence of low doses of deoxynivalenole on the level of this mycotoxin in pig serum. Medycyna Weterynaryjna, 60(5):534-536. Abstract from CABA 2004:103876.

## 14.0 References Considered But Not Cited

Bohm, J. and Razzazi, E. 2003. Effects of feeding deoxynivalenol contaminated wheat to piglets. Mycotoxin Res 19(2):176-179. Abstract from CABA 2004:128198.

Burch, D.G.S., and Roswell, C. 2001. The role of mycotoxins in PMWS—fact or fiction. Pig J, 48:142-147.

Danicke, S., Valenta, H., Klobasa, F., Doll, S., Ganter, M., and Flachowsky, G. 2004. Effects of graded levels of *Fusarium* toxin contaminated wheat in diets for fattening pigs on growth performance, nutrient digestibility, deoxynivalenol balance and clinical serum characteristics. Arch Anim Nutr, 58(1):1-17.

Danicke, S., Brussow, K.-P., Valenta, H., Ueberschar, K.-H., Tiemann, U., and Schollenberger, M. 2005. On the effects of graded levels of *Fusarium* toxin contaminated wheat in diets for gilts on feed intake, growth performance and metabolism of deoxynivalenol and zearalenone. Mol Nutr Food Res, 49(10):932-943.

Danicke, S., Brussow, K.P., Goyarts, T., Valenta, H., Ueberschar, K.H., and Tiemann, U. 2007. On the transfer of the *Fusarium* toxins deoxynivalenol (DON) and zearalenone (ZON) from the sow to the full-term piglet during the last third of gestation. Food Chem Toxicol, 45(9):1565-1574.

Dersjant-Li, Y., Verstegen, M.W.A., and Gerrits. W.J.J. 2003. The impact of low concentrations of aflatoxin, deoxynivalenol or fumonisin in diets on growing pigs and poultry. Nutr Res Rev, 16(2):223-239. Abstract from CABA 2004:27859.

Drochner, W., Schollenberger, M. Piepho, H.P., Götz, S., Lauber, U., Tafaj, M., Klobasa, F., Weiler, U., Claus, R., and Steffl, M. 2004. Serum IgA-promoting effects induced by feed loads containing isolated deoxynivalenol (DON) in growing piglets. J Toxicol Environ Health A, 67(13):1051-1067.

Gray, J.S., Bae, H.K., Li, J.C.B., Lau, A.S., and Pestka, J.J. 2008. Double-stranded RNA-activated protein kinase mediates induction of interleukin-8 expression by deoxynivalenol, Shiga toxin 1, and ricin in monocytes. Toxicol Sci, 105(2):322-330.

Hicks, L.R., Brown, D.R., Storch, R.H., and Bushway, R.J. 2000. Need to determine the relative developmental risks of *Fusarium* mycotoxin deoxynivalenol (DON) and benomyl (BEN) in wheat. Hum Ecol Risk Assess, 6(2):341-354.

Kim, E.-J., Jeong, S.-H., Cho, J.-H., Ku, H.-O., Pyo, H.-M., Kang, H.-G., and Choi, K.-H. 2008. Plasma haptoglobin and immunoglobulins as diagnostic indicators of deoxynivalenol intoxication. J Vet Sci, 9(3):257-266.

Rotter, B.A., Prelusky, D.B., and Thompson, B.K. 1996. The role of tryptophan in DON-induced feed rejection. J Environ Sci Health B, 31(6):1279-1288. Abstract from PubMed 8896360.

Severino, L., Luongo, D., Bergamo, P., Lucisano, A., and Rossi, M. 2006. Mycotoxins nivalenol and deoxynivalenol differentially modulate cytokine mRNA expression in Jurkat T cells. Cytokine, 36(1-2):75-82. Abstract from PubMed 17166736.

Sundstol Eriksen, G., and Pettersson, H. 2003. Lack of de-epoxidation of type B trichothecenes in incubates with human faeces. Food Addit Contam, 20(6):579-582. Abstract from MEDLINE 2003348756.

Swamy, H.V. L.N., Smith, T.K., MacDonald, E.J., and Sefton, A.E. 2001 abstr. Effects of feeding blends of grains naturally-contaminated with *Fusarium* mycotoxins on growth and brain regional neurochemistry of starter pigs and the efficacy of supplemental yeast cell wall polymer in detoxification. Abstract No. 442. J Dairy Sci, 84(Suppl. 1):106.

Tassis, P.D., Alexopoulos, C., Kritas, S.K., Tzika, E.D., Saoulidis, K., and Kyriakis, S.C. 2005. Mycotoxicosis of swine. Metabolism and toxicokinetics of their causative mycotoxins (Greek). Del Ellen Kteniatrikes Etaireias, 56(4):325-338. Abstract from CABA 2006:78637.

Tiemann, U., Brussow, K.P., Dannenberger, D., Jonas, L., Pohland, R., Jager, K., Danicke, S., and Hagemann, E. 2008. The effect of feeding a diet naturally contaminated with deoxynivalenol (DON) and zearalenone (ZON) on the spleen and liver of sow and fetus from day 35 to 70 of gestation. Toxicol Lett, 179(3):113-117. Abstract from PubMed 18550300.

Widestrand, J., Lundh, T., Pettersson, H., and Lindberg, J.E. 2003. A rapid and sensitive cytotoxicity assay for trichothecenes in cereal samples. Food Chem Toxicol, 41(10):1307-1313.

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## Appendix A: Units and Abbreviations

ºC = degrees Celsius
µg/g = microgram(s) per gram
µg/kg = microgram(s) per kilogram
µg/L = microgram(s) per liter
µg/$m^3$ = microgram(s) per cubic meter
µg/mL = microgram(s) per milliliter
µM = micromolar
µmol/kg = micromole(s) per kilogram
Ac-DON = acetyldeoxynivalenol
APC = aerobic plate counts
BrdU = 5-bromo-2´-deoxyuridine
bw = body weight
DAS = diacetoxyscirpenol
DON = deoxynivalenol
ECD = electron capture detection
ELISA = enzyme-linked immunosorbent assay
F = female(s)
FDA = Food and Drug Administration
FHB = *Fusarium* head blight
FusX = fusarenon X
g = gram(s)
g/mL = gram(s) per milliliter
GC = gas chromatography
GC-ECD = gas chromatography-electron capture detection
GIPSA = Grain Inspection, Packers and Stockyards Administration
h = hour(s)
HPLC = high performance liquid chromatography
IFI = internal *Fusarium* infection
Ig = immunoglobulin
IgA = immunoglobulin A
IgA-IC = immunoglobulin A immune complex
IgE = immunoglobulin E
IL = interleukin
i.p. = intraperitoneal(ly)
IR = infrared
JECFA = Joint FAO/WHO Expert Committee on Food Additives
kg = kilogram(s)
L = liter(s)
lb = pound(s)
LC = liquid chromatography
$LD_{50}$ = lethal dose for 50% of test animals
LPS = lipopolysaccharide
M = male(s)
MAPK = mitogen-activated protein kinase phosphorylation

mg/kg = milligram(s) per kilogram
$mg/m^3$ = milligram(s) per cubic meter
mg/mL = milligram(s) per milliliter
min = minute(s)
mL/kg = milliliter(s) per kilogram
mm = millimeter(s)
mM = millimolar
mmol = millimole(s)
mmol/kg = millimoles per kilogram
mo = month(s)
mol = mole(s)
mol. wt. = molecular weight
MS = mass spectrometry
MYC = mold and yeast count
ng/g = nanogram(s) per gram
NIV = nivalenol
NOAEL = no-observed-adverse-effect level
n.p. = not provided
NTP = National Toxicology Program
PMTDI = provisional maximum tolerable daily intake
ppb = parts per billion
ppm = parts per million
s.c. = subcutaneous(ly)
SCAR = sequence characterized amplified region
TLC = thin-layer chromatography
TNF = tumor necrosis factor
UV = ultraviolet
wk = week(s)

## Appendix B: Description of Search Strategy and Results

2004

A recent comprehensive literature review of DON by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2001 [http://www.inchem.org/documents/jecfa/jecmono/v47je05.htm]) covered the literature through 1999 with a partial representation of studies published in 2000 and one 2001 publication. The primary focus of the assigned literature search was on subsequently published literature on carcinogenicity and human exposure. The major literature search limited retrievals to publications from 2000-2004. The year 2000 publications retrieved by the search were compared to those already cited by JECFA (2001). Approximately 190 of the ~560 pre-2001 publications covered in PubMed were cited by JECFA (2001). An additional 168 PubMed records were found in the 2001-2004 publications, which indicates an overall growth of 30%.

The usual PubMed search strategy was deoxynivalenol OR vomitoxin OR (DON AND (mycotoxin* OR fung*)). The strategy was used to look for older publications on carcinogenesis and food contamination. To find additional information on the apoptotic and immunological effects of other trichothecenes, retrievals on DON were eliminated by combination of the DON terms using the NOT Boolean operator.

On March 19, 2004, files MEDLINE, CANCERLIT, NIOSHTIC, AGRICOLA, CABA, EMBASE, BIOTECHNO, BIOSIS, TOXCENTER, and NTIS were searched simultaneously on STN International. Some of the known acetylated deoxynivalenol derivatives were included. The edited online session below shows the keywords and strategy used. Year 2000 titles were kept separate to facilitate comparison with the year 2000 publications cited by JECFA (2001).

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L1          8584 S DEOXYNIVALENOL OR DESOXYNIVALENOL OR 51481-10-8
L2          4018 S DEHYDRONIVALENOL OR VOMITOXIN
L3          8688 S L1 OR L2
L4         31038 S DON
L5          2020 S L4 AND (MYCOTOXIN* OR TRICHOTHECENE* OR FUNG*)
L6          8748 S L3 OR L5
L7            60 S L6 NOT L3
L8          1290 S 88337-96-6 OR ACETYLDEOXYNIVALENOL OR ACETYLVOMITOXIN OR
                   ACETYL(W)4(W)DEOXYNIVALENOL
L9            41 S 51550-28-8 OR TRIACETYLDEOXYNIVALENOL
L10         8955 S L6 OR L8 OR L9
L11          437 S L10 AND 2000/PY
                  SET DUPORDER FILE
L12          170 DUP REM L11 (267 DUPLICATES REMOVED)
                  ANSWERS '1-27' FROM FILE MEDLINE
                  ANSWERS '28-44' FROM FILE AGRICOLA
                  ANSWERS '45-103' FROM FILE CABA
                  ANSWERS '104-109' FROM FILE EMBASE
                  ANSWERS '110-135' FROM FILE BIOSIS
                  ANSWERS '136-170' FROM FILE TOXCENTER
L13          170 SORT L12 1-170 TI
                  SAVE L13 X70PUB2000/A
                  SAVE L10 VOMITOXIN/Q
L14         1716 L10 AND (2001-2004)/PY
L15          561 DUP REM L14 (1155 DUPLICATES REMOVED)       Total L15 + L12
                  ANSWERS '1-161' FROM FILE MEDLINE                   188
                  ANSWERS '162-201' FROM FILE AGRICOLA                 57
                  ANSWERS '202-401' FROM FILE CABA                    259
                  ANSWERS '402-415' FROM FILE EMBASE                   20
                  ANSWER '416' FROM FILE BIOTECHNO                      1
                  ANSWERS '417-487' FROM FILE BIOSIS                   97
                  ANSWERS '488-561' FROM FILE TOXCENTER               109
L16          561 SORT L15 1-561 TI                                   731
                  SAVE L16 X7020012004/A
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Titles were examined and records were selected for printing in full if they covered contaminated grains and finished products, other potential routes for human exposure, or genotoxicity or repeated-dose toxicity studies. Studies of DON-induced immunomodulation represented a large fraction of the retrievals and were considered relevant to its potential for carcinogenicity based on the following statement by NTP (2002): "...[A]ltered hematopoietic (blood cell development) or immunologic events associated with exposure of humans or animals" may lead to "an increased incidence or severity of infectious disease or neoplasia because of the inability to respond adequately to the invading agent" (http://ntp-server.niehs.nih.gov/htdocs/IT-studies/about-Immunotoxicity.html). Therefore, full records of studies on DON-induced apoptosis in lymphocytes and other blood cells as well as studies of effects *in vivo* or *in vitro* on immunological factors such as cytokines and immunoglobulins were also printed. The immunotoxicity section in JECFA (2001), which reviewed numerous studies on immunomodulation by DON, provided important terms to look for in the new studies.

The STN International Registry file was searched to provide synonyms and CAS RNs of important derivatives before the major March 19 search. DART and EMIC were checked to see if any reproductive/developmental and genotoxicity studies had been overlooked. PubMed Central was searched to see if any recent publications were freely available electronically.

Limited searches of the Internet were done, primarily using the Google search engine.

2008

A comphrehensive literature review of DON was prepared in 2004. The primary focus of the previous document was to evaluate literature published subsequent to the literature review of DON by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2001a), which covered the literature through 1999 with a partial representation of studies published in 2000 and one 2001 publication. The primary focus of the 2008 literature search was on toxicity studies published since the preparation of the 2004 draft document (2004-2008). The year 2004 publications retrieved by the search were compared to those already cited by prior draft document.

On June 20, 2008, a PubMed search was conducted. The search strategy was deoxynivalenol OR vomitoxin OR dehydronivalenol OR (DON AND (mycotoxin* OR fung* OR trichothec*)) with publication dates from 2004 to 2008. The strategy yielded 382 references, 79 of which were published in 2004.

| | | |
|---|---|---|
| #1 | Search deoxynivalenol OR vomitoxin OR (DON AND (mycotoxin* OR fung*)) | 1105 |
| #2 | Search deoxynivalenol OR vomitoxin OR (DON AND (mycotoxin* OR fung*)) Limits: Publication Date from 2004 to 2008 | 382 |
| #12 | Search deoxynivalenol OR vomitoxin OR (DON AND (mycotoxin* OR fung* OR trichothec*)) Limits: Publication Date from 2004 to 2008 | 382 |
| #13 | Search deoxynivalenol OR vomitoxin OR dehydronivalenol OR (DON AND (mycotoxin* OR fung* OR trichothec*)) Limits: Publication Date from 2004 to 2008 Sort by: PublicationDate | 382 |

An initial search using the same terms used in 2004, without restriction to year, yielded 1105 results. Limiting the search to those articles published in 2004 or later and additional terms (see searches #2, #12, and #13) yielded the same number of hits, 382.

On June 23, 2008, files MEDLINE, AGRICOLA, CABA, EMBASE, BIOTECHNO, ESIOBASE, IPA, BIOSIS, TOXCENTER, FSTA, FROSTI, PASCAL, and NTIS were searched simultaneously on STN International. Some of the known acetylated deoxynivalenol derivatives were included. The edited online session below shows the keywords and strategy used. Titles were examined and records were selected for printing in full if they covered toxicity studies.

```
L1      4718 S (DEOXYNIVALENOL OR DESOXYNIVALENOL OR 51481-10-8) AND 2004-2008/PY
L2      1169 S (VOMITOXIN OR DEHYDRONIVALENOL) AND 2004-2008/PY
L3      4753 S L1 OR L2
L4      1755 S (DON AND (MYCOTOXIN* OR FUNG* OR TRICHOTHEC*)) AND (2004-2008)/PY
L5      4794 S L3 OR L4
L6      617 S (ACETYLDEOXYNIVALENOL OR ACET OR ACETYLVOMITOXIN OR 50722-38-8 OR
        8837-96-6) AND (2004-2008)/PY
L7      2 S (51550-28-8 OR TRIACETYLDEOXYNIVALENOL) AND (2004-2008)/PY
L8      4956 S L5 OR L6 OR L7
L9      1438 S DUP REMOVE L8 (3518 DUPLICATES REMOVED)
          ANSWERS '1-363' FROM FILE MEDLINE
          ANSWERS '364-511' FROM FILE AGRICOLA
          ANSWERS '512-864' FROM FILE CABA
          ANSWERS '865-906' FROM FILE EMBASE
          ANSWERS '907-925' FROM FILE ESBIOBASE
          ANSWERS '926-927' FROM FILE IPA
          ANSWERS '928-1086' FROM FILE BIOSIS
          ANSWERS '1087-1344' FROM FILE TOXCENTER
          ANSWERS '1345-1378' FROM FILE FSTA
          ANSWERS '1379-1408' FROM FILE FROSTI
          ANSWERS '1409-1438' FROM FILE PASCAL
L10     1438 SORT L9 1-1438 TI
```

On June 24, 2008, a PubMed search was performed for structure-activity relationship studies. The edited PubMed search strategies used are provided below.

| | | |
|---|---|---|
| #1 | Search nivalenol OR 23282-20-4 Limits: Publication Date from 2004 to 2008 | 91 |
| #7 | Search fusarenon OR fusarenone OR (nivalenol AND (acetate OR monoacetate)) OR 23255-69-8 Limits: Publication Date from 2004 to 2008 | 30 |
| #11 | Search acetyldeoxynivalenol OR 50722-38-8 OR 88337-96-6 Limits: Publication Date from 2004 to 2008 | 38 |
| #13 | Search acetyldeoxynivalenol OR 51550-28-8 OR triacetyldeoxynivalenol OR (vomitoxin AND triacetoxy) Limits: Publication Date from 2004 to 2008 | 38 |
| #14 | Search #13 NOT #11 Limits: Publication Date from 2004 to 2008 | 0 |
| #15 | Search acetyl AND deoxynivalenol Limits: Publication Date from 2004 to 2008 | 27 |
| #16 | Search #15 NOT #13 Limits: Publication Date from 2004 to 2008 | 24 |

The Google Scholar search engine was also used. The search strategy was the following: "deoxynivalenol OR vomitoxin OR dehydronivalenol" limited to the years 2004-2008. A total of 2900 hits returned and all were reviewed for relevance.

2009

On February 16, 2009, files MEDLINE, AGRICOLA, CABA, IPA, BIOSIS, TOXCENTER, FSTA, FROSTI, EMBASE, ESBIOBASE, and BIOTECHNO were searched simultaneously on STN International. Essentially, the same strategies had been used on June 23, 2008, and August 25, 2008, to update the toxicology information. (The NTIS and PASCAL databases were included in the June search, and FSTA and FROSTI were not used in either 2008 search. The strategies did not include the synonym in L5. The CAS Registry Number in L4 was not used in the June 2008 search.) Result totals in June 2008 August 2008, and February 2009 were 1438, 738, and 1920, respectively. Selection totals were 61, 19, and 251, respectively. EndNote was used to eliminate toxicology duplicates before the 2009 selections. The wider selection in 2009

provided information for updating the report environmental and exposure sections. The search history for the 2009 online session is reproduced below.

```
L1        15614 S DEOXYNIVALENOL OR DESOXYNIVALENOL OR 51481-10-8
L2         5487 S DEHYDRONIVALENOL OR VOMITOXIN
L3         8415 S DON AND (MYCOTOXIN? OR TRICHOTHECENE? OR FUNG?)
L4         2012 S 88337-96-6 OR ACETYLDEOXYNIVALENOL OR ACETYLVOMITOXIN
L5          959 S ACETYL(W)4(W)DEOXYNIVALENOL OR 50722-38-8
L6           45 S 51550-28-8 OR TRIACETYLDEOXYNIVALENOL
L7        17301 S L1-L6
L8         6009 S L7 AND (2004-2009)/PY
                SET DUPORDER FILE
L9         1920 DUP REM L8 (4089 DUPLICATES REMOVED)
L10        1920 SORT L9 1-1920 TI
                SAVE L9 X70RAW/A
                SAVE L10 X70SORT/A
```